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Study of the Distribution and Variation of the Herbicide Atrazine in Finished Drinking Water at a Small Community Water System in Kentucky

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STUDY OF THE DISTRIBUTION AND VARIATION OF THE HERBICIDE ATRAZINE IN FINISHED DRINKING WATER AT A SMALL COMMUNITY WATER SYSTEM IN KENTUCKY

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ABSTRACT

This study examines the variation in the distribution of the concentration of atrazine, a triazine herbicide used in Kentucky to control weeds primarily in corn fields. Atrazine is known to have carcinogenic properties and is an endocrine disruptor in aquatic species even at low concentrations. Atrazine has the ability to be transported through the environment into water bodies due to its physical and chemical properties favoring its occurrence and distribution. Raw and Finished drinking water samples were collected from the Lewisburg water treatment plant which derives its drinking water supplies from a source water intake namely Spa Lake, which has a direct run-off from the fields with abundant atrazine application. Atrazine was analyzed in the collected water samples and was found in both the raw and finished drinking water in concentrations exceeding the Maximum Contaminant Level (MCL) of 3 ppb as established by the US Environmental Protection Agency (US E.P.A).The Safe Drinking Water Act (SDWA) methodology requires quarterly sampling strategy to be implemented by all small community water systems to monitor and control the concentrations of atrazine in finished drinking water. The presence of missing high concentrations of atrazine in finished water which are not measured in the regular quarterly sampling protocol is of concern for exposures and protection of public health and environment.

Chapter-1

INTRODUCTION

Problem Definition

Water for potable use is "finite," as fresh water resources are limited, and the same quantity of water is being recycled again and again in the water cycle. The various sources of fresh and consumable water are groundwater, rain water, ponds, lakes, reservoirs, streams and rivers. According to the United Nations Environment Program, there is a necessity for safe drinking water and adequate sanitation as today more than two billion people lack access to safe drinking water worldwide(1). In recent years there has been a significant increase in the use of herbicides and pesticides in the production of corn and soybeans, and other agricultural crops (2). The increased reliability on the efficiency and efficacy of these substances has caused many environmental threats, affecting not only the ecosystem in general but the human population and its health as well (3). The bioaccumulation of these organic compounds in various stages of the food web is of great concern due to the increased risk of biomagnification and health effects (4).

Atrazine, a triazine herbicide, used for controlling weeds in corn, sorghum, sugarcane and wheat fields came into wide usage in the 1970s. This product was first introduced in 1958, and it has increased steadily over the past 44 years. In 2002 approximately 76 million pounds of Atrazine were applied in the United States (6). According to the EPA's National Survey of Pesticides in Drinking Water Wells, atrazine was found to be the second most frequently detected pesticide (15). Atrazine is

transported to water bodies by run-off from fields, in rainfall, due to misapplication, and improper disposal. This event is of great concern when those water bodies happen to be source water for drinking water supplies, as source water contamination may increase the risk of exposure through consumption. This ingestion forms a major and a direct route of exposure. Atrazine, being a potential carcinogen to humans, can then be transported from the environment to the human body through drinking water.

Atrazine is currently regulated by the Safe Drinking Water Act (SDWA) as a synthetic organic compound (U.S Environmental Protection Agency). A Maximum Contaminant Level (MCL) of 3 parts per billion (ppb) was established by the U.S Environmental Protection Agency (EPA). Under the SDWA, community water systems are required to monitor for atrazine and maintain a yearly average below the MCL (6). According to EPA's Revised Risk Assessment, twenty-nine community water systems in the United States had intermediate to chronic term exposures to atrazine and its metabolites that exceeded levels of concern (7). One such water system, located in Lewisburg, Kentucky, whose raw water supply is Spa Lake, was identified as having levels above the MCL in raw source water and finished drinking water (12).

Atrazine as an Environmental Contaminant

Atrazine has endocrine-disrupting effects on aquatic organisms, such as frogs and fish (7). It has been found to cause demasculinization of frogs even at concentrations below the Maximum Contaminant Levels (MCL) and as low at 0.1 ppb. Atrazine causes reduction in olfactory-mediated endocrine functions in Salmonids (8). EPA classified the human carcinogenic potential of atrazine as "possible," or category "C" under the cancer

assessment guidelines issued in 1986. However, the role of atrazine as a potential causative agent of increased incidences of breast and prostate cancers in communities consuming atrazine-contaminated water was not clearly defined. A study conducted by the University of Kentucky attempted to identify the relationship between the increased incidence of breast cancer and triazine herbicide exposures in Kentucky Counties. It was concluded that the results revealed a statistically significant increase in breast cancer with medium and high levels of triazine exposure (odds ratio (OR) =1.14, $p<0.0001$ and OR=1.2, $p<0.001$, respectively) (9). Although, there is clear evidence of the real potential of atrazine to directly cause adverse effects to aquatic biota, the human health effects of atrazine are still being investigated and documented.

Research Objective:

This study is an assessment of the occurrence and variation of atrazine in the finished water of a community water supply, Lewisburg, Kentucky, and assesses the current method of quarterly sampling to determine noncompliance with the SDWA. Although the focus of this research is on the occurrence and variation of atrazine in finished drinking water, the use of immunoassay monitoring of atrazine was investigated as a tool for developing agricultural partnerships. We assessed whether scientific data could be used to increase public health awareness in a farming community. Another potential benefit of this model is translating watershed level impacts and management practices to the state and federal level. Understanding the occurrence and variation of atrazine through assessment should foster community partnerships and ultimately lead to public health and environmental protection. The primary research question investigated

was as follows: Can the current SDWA methodology for small water systems to test for atrazine contamination in finished drinking water on a quarterly basis provide an accurate representation of the annual variation in the occurrence of atrazine?

This study is applicable to community water systems that have source water supplies in agricultural watersheds where corn is produced and atrazine is applied. Specifically, it illustrates how assessment, utilizing the immunoassay method of atrazine detection, can be used to elucidate the annual occurrence and variation of this herbicide. Further, this study defines the need for economical means to measure atrazine concentrations and other herbicides/pesticides in finished water of small rural water systems. This study is specific to Kentucky in that the study area was within a karstic agricultural basin. Identified limitations of this research include the following:

1. The site for research was selected based on the data available as to the application of atrazine and the history of contamination of a community water supply. Selection was not at random. Therefore, a purposive sampling strategy was used.
2. The analysis of the water samples for atrazine utilized the technique of immunoassay due to the costs incurred in the process. The analysis did not utilize the more sophisticated gas chromatography (GC) for this research. However, the technique is adequate to detect levels of concern in this study.
3. The test protocol used here detected for all triazines, and just not atrazine. This testing can result in "false positives."
4. An absence or a delay in sampling due to operator availability or time constraints, which could result in slight variations in the data being analyzed.

5. Sampling bottles were broken in transport/ storage for a very small portion of the samples.
6. On occasion the levels of atrazine in finished water exceeded that of the raw water at the Lewisburg Water Treatment Plant (LWTP).
7. The range of detection by the Immunoassay method lies between 0.04 and 5.0 ppb. So, there is a possibility that the exact concentration above the detection limit may not be recorded and/or dilutions to detect high values caused errors.
8. A concentration below 0.04 ppb was recorded as non-detectable by the Immunoassay method. In such circumstances, the lowest value shown on the reading is recorded.
9. Potential improper handling of the sampling bottles by the LWTP operators and laboratory technicians.
10. Cross-contamination between the raw water and finished water in the water treatment plant.
11. The number of samples collected each year is not the same.

Chapter-2

BACKGROUND

General Description

The environmental fate of atrazine is very complex. It can be present in soil with a half-life of more than one year. Atrazine is considered to be mobile and is transported throughout the environment due to its solubility in water. It is transported to surface water via run-off, spray drift and atmospheric transport. Atrazine has a history of being detected in rainfall, groundwater, and surface waters. These characteristics make atrazine a potential contaminant of all types of freshwater bodies. The resistance of atrazine to abiotic hydrolysis and direct aqueous photolysis and its moderate susceptibility to degradation in soil supports the fact that atrazine doesn't undergo rapid degradation on foliage. Atrazine has been observed to be more persistent in a colder climate, suggesting the site specific nature of the product (10). The result is significant variation in the persistence of atrazine in the environment and thereby may have affected the study results. Atrazine is known to be relatively persistent in soils with a half life ranging from 4 to 57 weeks (22). However, limited data exists as to the soil conditions and watershed processes that may enhance transport. The above discussion describes the complexity of the environmental transport of atrazine stressing the importance of studies in this field to determine and identify the reasons for the persistence of concentrations in drinking water which could potentially lead to human health concerns.

Product Identity and Uses

Commercial Atrazine should be at least 92% pure according to the Food and Agricultural Organization (FAO). But most of the available atrazine products are 95% pure, almost 3% above the ideal state. Annually in Kentucky, more than 1 million pounds of atrazine are applied (9). This quantity of application is a point of concern in terms of environmental protection and health preservation, as expanded use results in a ubiquitous distribution of atrazine in the environment. Some of the Trade Names/Synonyms for this product are as follows: Aatrex; Actinite PK; Akticon; Argezin; Atazinax; Atranex; Atrataf; Atred; Candex; Cekuzina-T; Chromozin; Crisatrina; Cyazin; Fenamin; Fenatrol; Gesaprim; Griffex; Hungazin; Inakor; Pitezin; Primatol; Radazin; Strazine; Vectal; Weedex A; Wonuk; Zeapos; Zeazine (20).

Physical and Chemical Properties:

Atrazine is a colorless crystalline powder with a low vapor pressure. It has a melting point of 175-177 degrees Celsius. It is readily soluble in many organic solvents such as methanol, diethyl ether, dimethyl sulfoxide, chloroform, etc. It is slightly soluble in water, yet compared to other synthetic organic compounds it is quite soluble. Atrazine is considered to be stable in the dry state, but is hydrolyzed in acid or alkaline solutions (10). Two primary characteristics of atrazine that increase risks of contamination of community water supplies are its mobility and resistance to metabolism (9).

Atrazine Exposure, Metabolism and Excretion

Atrazine is easily absorbed from the gastrointestinal tract but only to a certain extent through the skin (10). Studies on rats showed that this herbicide and its metabolites bind effectively to the red blood cells and to the tissues of some of the major organs. Atrazine is rapidly eliminated from the body through urine and feces. Cardiac toxicity was observed in dogs after long-term oral administration of atrazine. Some of the effects observed in rats and mice, after experimental studies, were reduced food intake, decreased weight gain, and toxic effects, such as muscle and retinal degeneration, necrosis of the liver, and hematological effects. In addition, an increase in mammary tumors was observed in rats (10).

The potential of atrazine to cause carcinogenesis in humans is still under research. It is known that xenoestrogens promote cancer by enhancing the production of genotoxic estrogens and mutations in cells. The fact that atrazine is a dubbed estrogen increases the importance of exploring the possibility of the carcinogenic potential of triazine herbicides. Exposure to excess estrogen is considered to be a risk factor for the development of breast cancer (9). Since atrazine has estrogenic properties, there is a possibility for it to cause hormonal imbalance that may result in an increased chronic incidence of breast cancer by consumption of atrazine-contaminated water. Atrazine was also related to an elevated incidence of prostate cancer in men. This observation was made at the St.Gabriel atrazine plant in Louisiana where the employees had markedly elevated rates of prostate cancer, which were significantly higher than the statewide average (11). The National Institute of Environmental Health Sciences has been conducting significant research for determining the role of environmental estrogens and

toxicological substances in initiating and/or promoting breast cancer. It has been observed that the incidence of breast cancer has increased during the years of increased organochlorine use in the United States. The U.S.E.P.A has classified atrazine as a "possible" human carcinogen, as it was placed in category "C" under the cancer assessment guidelines (6).

Atrazine has a potential to cause many acute health effects. These occur especially when the exposures are above the MCL. Some of the acute health effects include muscle spasms, congestion of heart, lungs and kidneys, hypotension, antidiuresis, adrenal degeneration, etc (20). Documented chronic health effects in humans due to the consumption of atrazine contaminated water are weight loss, mammary tumors, muscle degeneration, cardiovascular damage, and retinal degeneration. Atrazine is thought to have carcinogenic potential when there are lifetime exposures above the MCL (20). It can be manifested as cancer of various organs in males and females.

Safe Drinking Water Act Regulations

The U.S EPA has determined the safe levels of chemicals in drinking water with an objective to monitor the health risks caused by exposure to atrazine and other chemicals. These are called Maximum Contaminant Level Goals (MCLG). The MCLG for atrazine was set at 3 ppb. Based on this standard, the MCL for atrazine was set at the same level. This setting was done to enforce an atrazine standard in drinking water, requiring drinking water systems to effectively treat and remove this contaminant from drinking water, or limit the occurrence in source water through best management

practices. Under the National Primary Drinking Water Regulations, it is mandatory for all public water systems to abide by this regulation (15).

The Safe Drinking Water Act (SDWA) was enacted by the U.S Congress in 1974. This action was primarily taken to manage, monitor, and prevent potential contaminants from reaching groundwater. "Section 1447 of the SDWA states that each federal agency having jurisdiction over a federally owned or maintained public water system must comply with all federal, state, and local requirements; administrative authorities; processes and sanctions regarding the provision of safe drinking water"(16). Also, public water systems are authorized under sections 1412, 1414, and 1445(a) of the SDWA for drinking water regulations and specific operating procedures (16). The SDWA authorizes the U.S.EPA to set national health-based standards to drinking water in order to protect against both man-made and naturally occurring contaminants in drinking water (16). The 1996 amendment of the SDWA focused primarily on the recognition and development of source water protection, funding for small water systems, operator training and education, and public awareness and information.

One of the greatest steps in the amendment was to lay special emphasis on capacity development of Small Water Systems in terms of their managerial, financial and technical abilities (16). This capacity development would lead to the ability of these systems to become self-reliable in protecting and preserving water quality through the delivery of safe drinking water to the public. The monitoring for Ground/Surface Water Sources includes sampling and analysis for atrazine at an initial frequency of four

quarterly samples every three years. This procedure is followed by a repeat frequency of sampling. If no detections are found in the initial round, then two quarterly samples are to be collected per year if the water system is serving a population of more than 3300. For yet smaller systems, one sample per three years is to be collected. If triggers are detected at greater than 0.001 mg/L, the initial sampling frequency is to be applied for the water system.

Drinking Water Treatment Technologies for Atrazine

Many small rural drinking water systems do not have enough capability and infrastructure to treat drinking water to eliminate atrazine. The conventional water treatment techniques do not effectively remove this herbicide from drinking water. On the other hand, atrazine in drinking water has the property to form transformation products (23). Removal of atrazine from ground and drinking water requires expensive chemical adsorption procedures. The typical procedure to remove atrazine is the use of activated charcoal. But, the federal mandate requires that potable drinking water should not contain atrazine above the MCL, which is 3ppb. In view of this issue, research has been done at various levels to develop an effective and inexpensive method for atrazine removal from drinking water. Studies were done to test the effectiveness of the purified atrazine chlorohydrolase enzyme to remove atrazine from raw, contaminated, surface drinking water supplies (18). Powdered activated carbon (PAC) filtration, granular activated carbon (GAC) filtration, and reverse osmosis have been demonstrated to be highly effective in treating drinking water for the complete and effective removal of

atrazine. Through various studies, it was found that PAC is the most common method used as it can be used in concert with conventional water treatment systems with no additional investments and infrastructure establishments (19) an anecdote to our study may be that PAC did not reduce levels significantly. The best available technology for the treatment of atrazine in drinking water has been shown to be Granular Activated Charcoal (GAC) (20). The Lewisburg Water Treatment Plant (LWTP) at Lewisburg, Kentucky used Powdered Activated Carbon (PAC) in its treatment process in an attempt to remove atrazine from raw water.

Chapter-3

METHODOLOGY

The research design used for this study included the monitoring of atrazine in the raw and finished water of the LWTP, assessing the weekly to biweekly occurrence and variation of atrazine in a rural community water supply in comparison to the strategy of quarterly sampling for compliance, and the evaluation of the assessment of atrazine as a tool for public health and source water protection. Additionally, we investigated assessment as a tool for public awareness about the land use practices that may contribute atrazine. Primary emphasis of this research related to the occurrence and variation of atrazine according to the following hypothesis:

Hypothesis Research Question: By stating the primary research question a supporting null hypothesis was developed. The primary research question was: Can the current SDWA methodology for small water systems to test for atrazine contamination in finished drinking water on a quarterly basis provide an accurate representation of the annual variation in the distribution of atrazine?

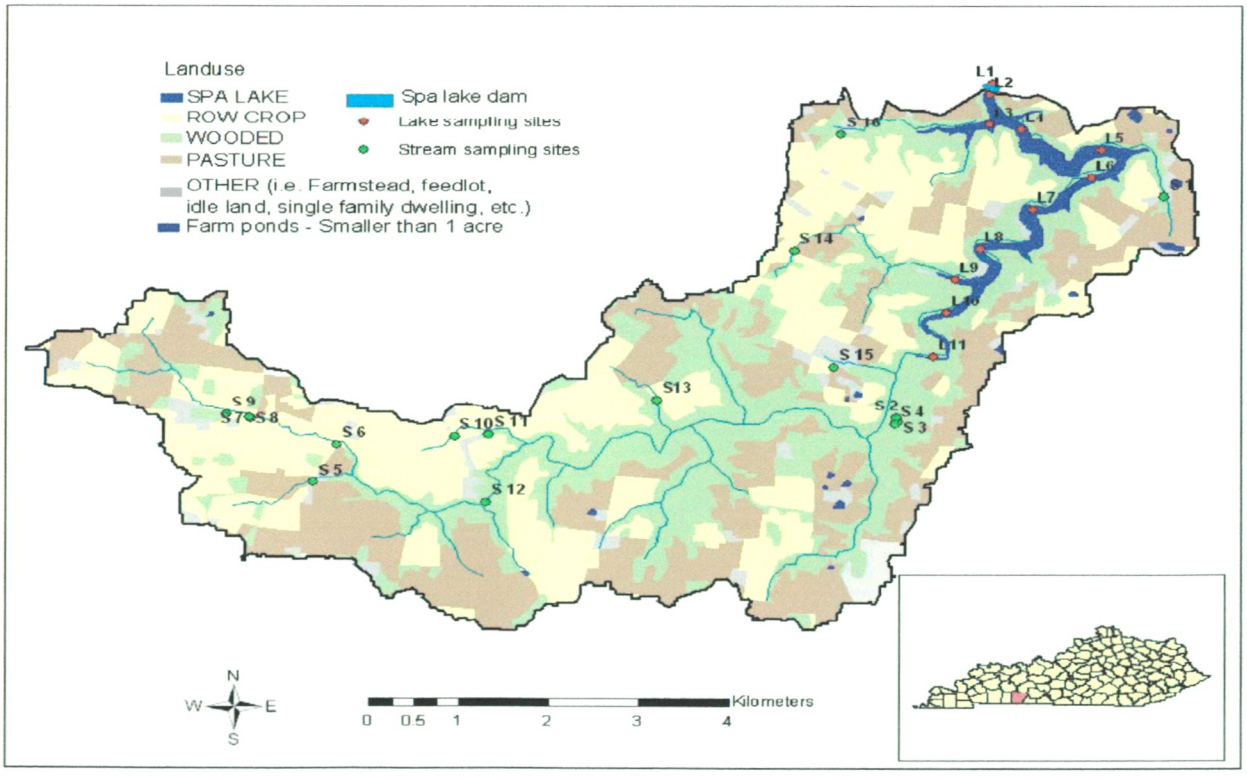
Null Hypothesis: The current methodology for small water systems to test for atrazine contamination in finished drinking water on a quarterly basis does provide an accurate representation of the annual variation in the distribution of atrazine.

Alternate Hypothesis: The current methodology for small water systems to test for atrazine contamination in finished drinking water on a quarterly basis does not provide an accurate representation of the annual variation in the distribution of atrazine.

Site Description:

Spa Lake is located in Logan County, Kentucky. Its watershed is spread over Logan and Todd Counties, including the southwest of the City of Lewisburg. Spa Lake was constructed in 1972 to serve not only for flood control, but also for public water supply for the city of Lewisburg. This agricultural watershed is dominated by cropland, pasture, and deciduous forests (14). This impoundment receives storm water runoff that contains quantities of atrazine at levels high enough to contaminate the source water supply (Fig.1) (24).

Figure 1: Spa Lake Watershed



Sampling Procedure:

The sampling procedure involved the weekly collection of raw and finished water samples from the LWTP. Also, water samples were collected from designated sites in the watershed (Lake and Stream sites) at a frequency of once per month. A duplicate sample was collected for every ten samples. These water samples were "grab samples." For the reservoir sites, samples were collected at various depths at each sampling location.

The water samples were collected in 40ml amber glass VOA bottles. The bottle was rinsed three times prior to the collection of an atrazine sample. The rinse water was dumped away from the sample site. While collecting the water sample, the VOA was faced upstream and the cap should be replaced underwater to prevent air bubbles in the container. This procedure was followed to avoid the escape of atrazine through the residual air between the water level and the cap of the sampling bottle. The bottles were preserved in a container at 2-8 degrees Celsius till they reached the CWRS laboratory. They were then transferred to a refrigerator and preserved at the same temperature until analyzed. The analysis was done within 14 days of the date of sample collection as per the EPA method for the analysis. If a longer holding time was required then they were preserved by adding sulfuric acid to a pH below 2.0(14).

Analysis of the Atrazine Water Samples:

The collected samples were analyzed at the CWRS laboratory within fourteen days of collection. The samples were analyzed using Strategic Diagnostics Inc. (SDI) RPA-I RaPID Analyzer and Atrazine Rapid Assay Test Kits (Appendix--C). The sample to be tested was added, along with an enzyme conjugate, to a disposable test tube,

followed by paramagnetic particles with antibodies specific to atrazine attached. Both the atrazine (which may be in the sample) and the enzyme labeled atrazine (the enzyme conjugate) compete for antibody binding sites on the magnetic particles. At the end of an incubation period, a magnetic field was applied to hold the paramagnetic particles (with atrazine and labeled atrazine analog bound to the antibodies on the particles, in proportion to their original concentration) in the tube and allow the unbound reagents to be decanted. After decanting, the particles were washed with Washing Solution.

The presence of atrazine was detected by adding the enzyme substrate and the chromogen. The enzyme labeled atrazine analog bound to the atrazine antibody catalyzes the conversion of the substrate/chromogen mixture to a colored product. After an incubation period, the reaction was stopped and stabilized by the addition of an acid. Since the labeled atrazine (conjugate) was in competition with the labeled atrazine (sample) for the antibody sites, the color developed was inversely proportional to the concentration of atrazine in the sample. The determination of the atrazine level in an unknown sample was interpreted by using a spectrophotometer. The spectrophotometer works on the principle of optical density at a wavelength of 450nm. The data was printed in ppb (parts per billion). This method was very convenient, inexpensive and reliable for analyzing atrazine samples (13).

GC techniques are considered to be a more sophisticated method for analysis of water samples for atrazine. But the limitations to its use were cost and instrument availability. However, the results obtained by using that method would be more reliable as they have less error on high and low scales of concentrations of atrazine.

Data Analysis:

Data were collected over a four year period, from May 1999 to April 2003, at the LWTP. The data were recorded on the computer using Microsoft Excel Spread Sheets and stored in the CWRS database. The data were statistically analyzed using S-Plus software and Environmental Stats module (21). Data for the LWTP finished water were analyzed by comparing atrazine concentrations for annual datasets with that of individual quarterly datasets for each year.

The quarterly sampling strategy was devised for each year by dividing it into equal sampling intervals in terms of time duration between the collection of the first sample and the next. Each year was divided into sampling patterns. Three patterns for quarterly sampling were identified in each year. The first pattern represents the months January, April, July, and October. The second pattern represents the months February, May, August, and November. The third pattern represents the months March, June, September, and December. The current methodology which calls for quarterly sampling in a year is taken as the basis for this strategy in statistical analysis and representation. These three sampling patterns were identified for each of the three study periods 2000, 2001, and 2002. The data for the sampling patterns was derived from the first sample for each month which was considered to be the first week's sample of each month for each year. Data was also obtained from the third sample of each month which amounted to third week's sample of each month for each study period. Data for the highest concentration in each month for all the three study periods was identified and segregated into quarterly patterns for analysis and comparison with the regular quarterly patterns for

each year. By observing the variation in the data, the hypothesis could be tested and a verification of the research question could be made.

Chapter 4

RESULTS

The results of this study obtained by analyzing water samples from the LWTP are included in this section. Data collected for four years from May 1999 to April 2003 are presented in tabular form in Appendix--A (Tables 1, 2, 3, and 4). Graphical analysis provided a means to assess trends in the data from May 1999 to April 2003. Results of statistical analysis provided a means to assess the distribution of the data and test the hypothesis previously stated.

Distribution and Occurrence of Atrazine

The data from May 1999 to April 2000, which shows the distribution of the concentration of atrazine over a period of four years, are presented in Figure 2. These data were divided into three study periods of one-year each. The study periods were the years 2000, 2001, and 2002. The data collected from January to December of each year, for all three years, is taken into consideration for this study to assess the annual occurrence and variation (Figure 3, 4, and 5). The data are included in Appendix--A (Tables 5, 6, and 7). Raw water concentrations are shown for comparative analysis to assess the potential removal due to water treatment processes. Also, data are provided for the study period to identify trends in the distribution of atrazine in finished drinking water.

Figure 2: Atrazine Concentration in Raw and Finished Drinking Water at Lewisburg Water Treatment Plant, May 1999 - April 2003

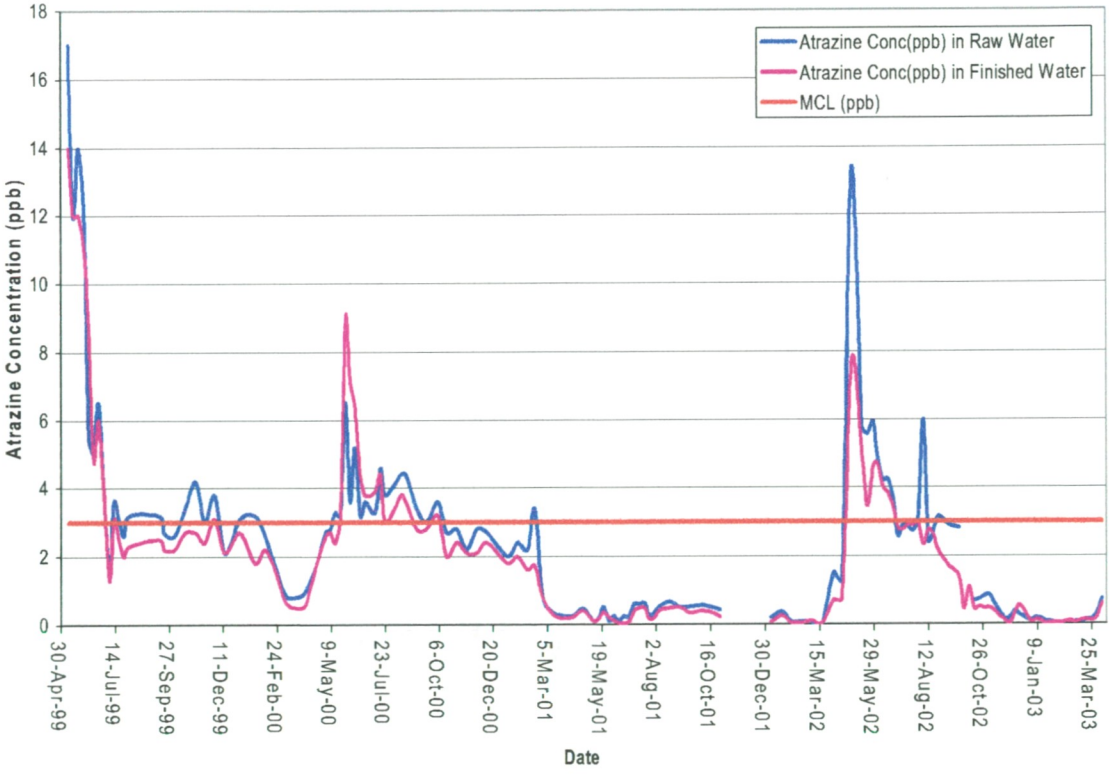


Figure 3: Atrazine Concentration in Raw and Finished Drinking Water at Lewisburg Water Treatment Plant, January 2000 - December 2000

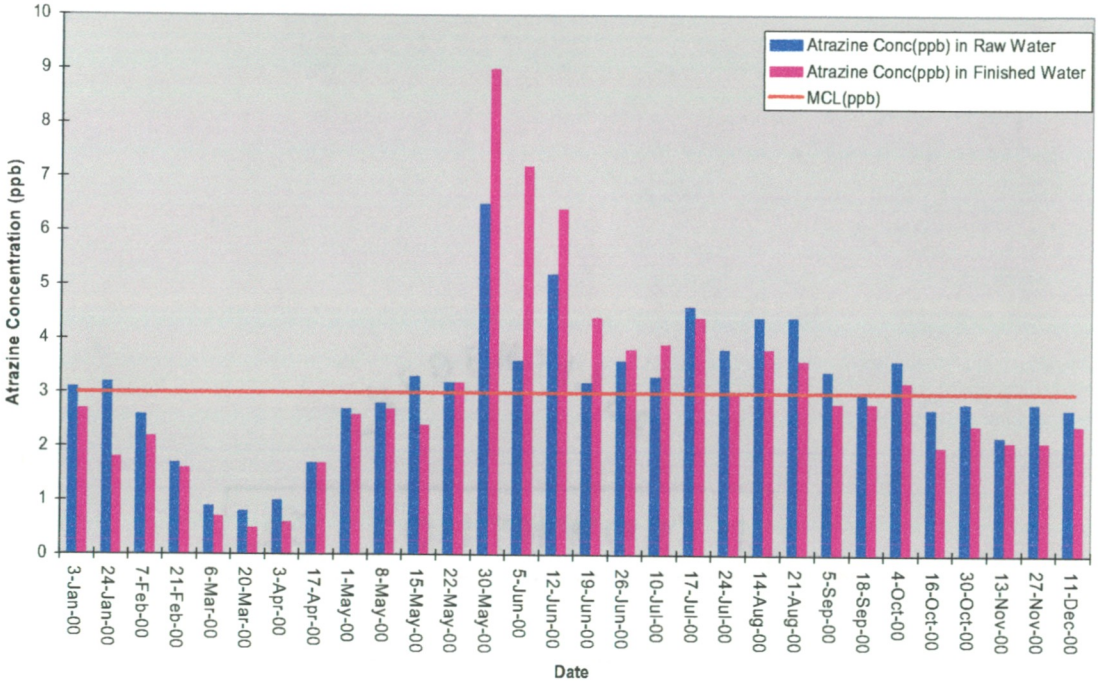


Figure 4: Atrazine Concentration in Raw and Finished Water at Lewisburg Water Treatment Plant, January 2001 - December 2001

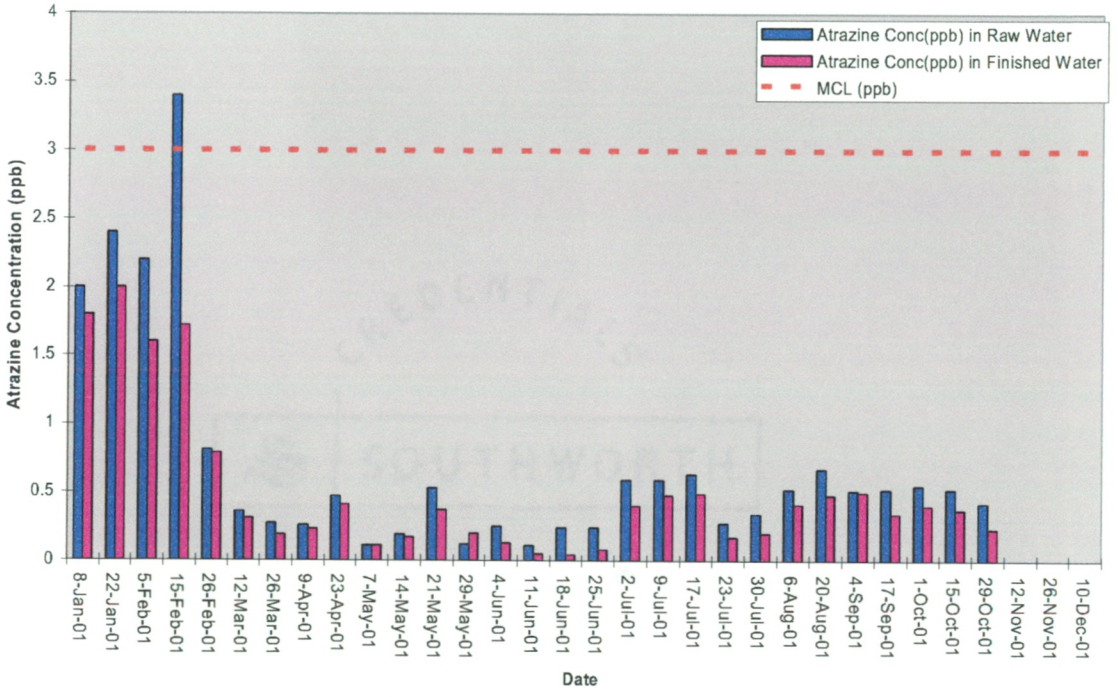
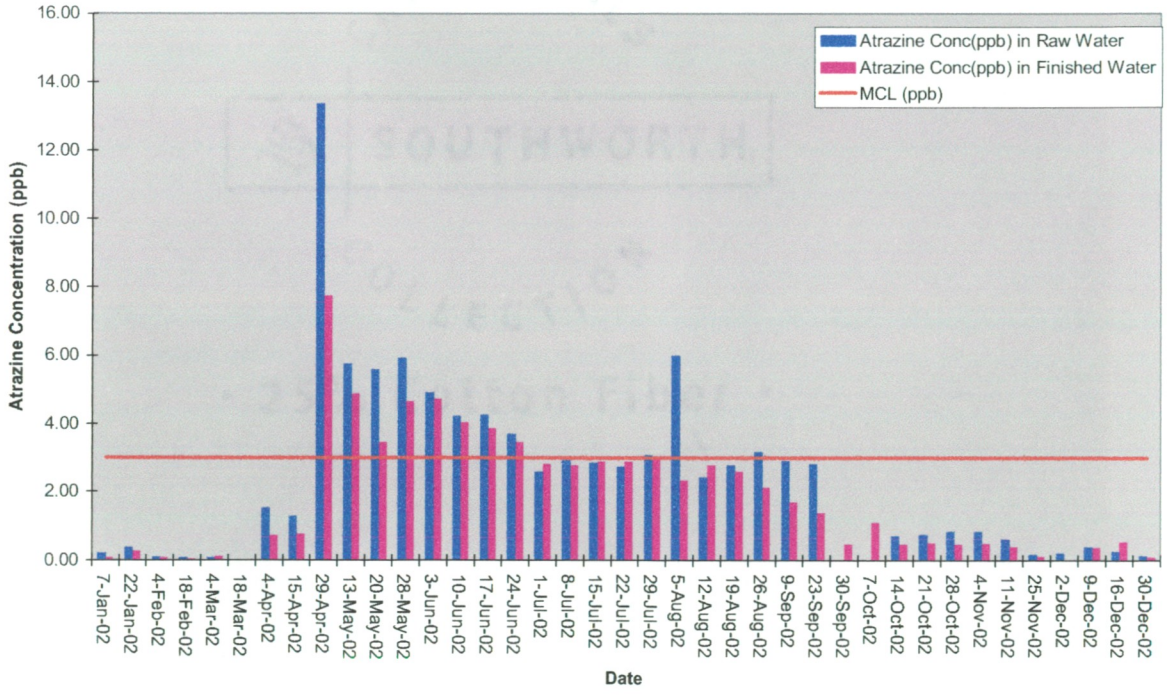


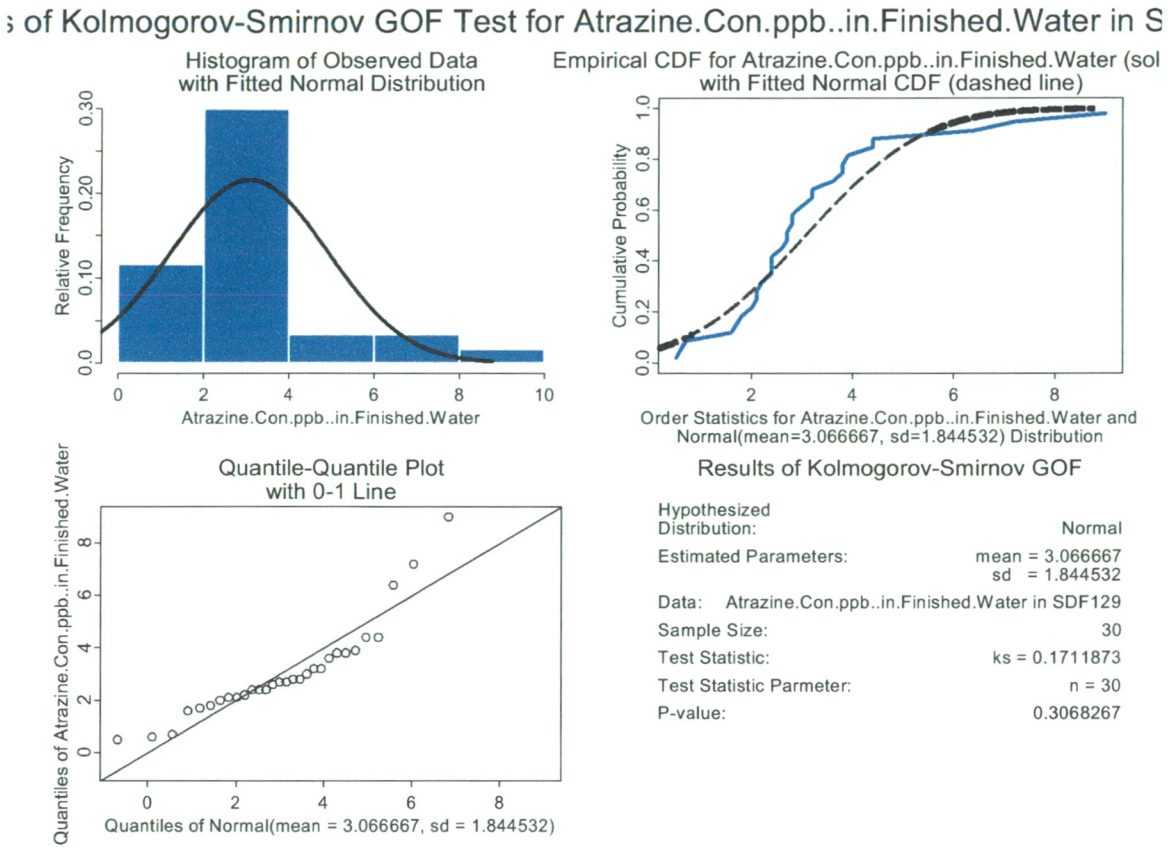
Figure 5: Atrazine Concentration in Raw and Finished Water at Lewisburg Water Treatment Plant, January 2002 - December 2002



Statistical Analysis of Annual Distribution

Annual atrazine data for the study periods of 2000, 2001, and 2002 were tested for normality. This testing was done to assess the annual distributions and compare these distributions from year to year. The analyses are graphically represented in the Figures 5, 6, and 7. The yearly data were first tested and analyzed and tested for normality. Thus, the data was tested for a specific distribution pattern. Analysis of the data for the three study periods was performed with the S-Plus® statistical system using the Kolmogorov-Smirnov (KS) goodness-of-fit test (21). This test was done to compare a set of samples with a specified probability distribution. Likewise, the analysis was used to ascertain statistical differences between years. A goodness-of-fit-test may be used to test the null hypothesis that the data come from a specific distribution, or to test the more general null hypothesis that the data come from a particular family of distributions.

Figure 6: Kolmogorov-Smirnov goodness-of-fit test for the Study Period 2000

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Results of Goodness-of-Fit Test
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Test Method:                    Kolmogorov-Smirnov GOF

Hypothesized Distribution:      Normal

Estimated Parameter(s):         mean = 3.066667
                                sd   = 1.844532

Estimation Method:

Data:                           Atrazine.Con.ppb...In.Finished.Water in SDF129

Sample Size:                    30

Test Statistic:                 ks = 0.1711873

Test Statistic Parameter:       n = 30

P-value:                        0.3068267

Alternative Hypothesis:         True cdf does not equal the
                                Normal Distribution.
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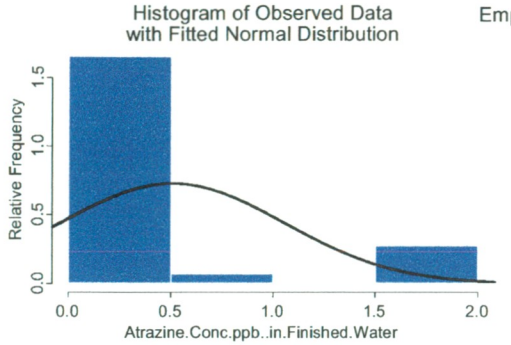
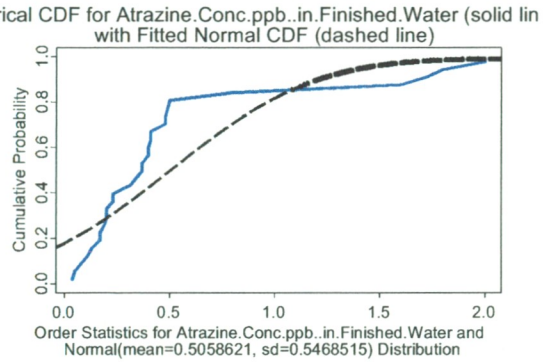
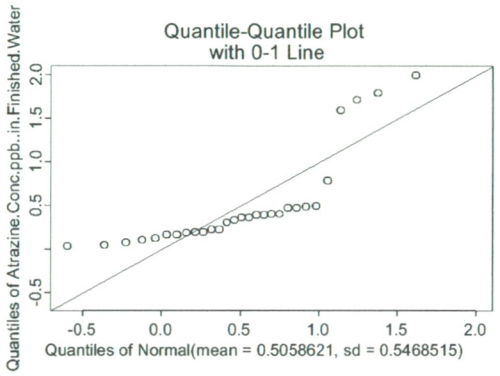
Figure 7: Kolmogorov-Smirnov goodness-of-fit test for the Study Period 2001

of Kolmogorov-Smirnov GOF Test for Atrazine.Conc.ppb..in.Finished.Water in SDF131





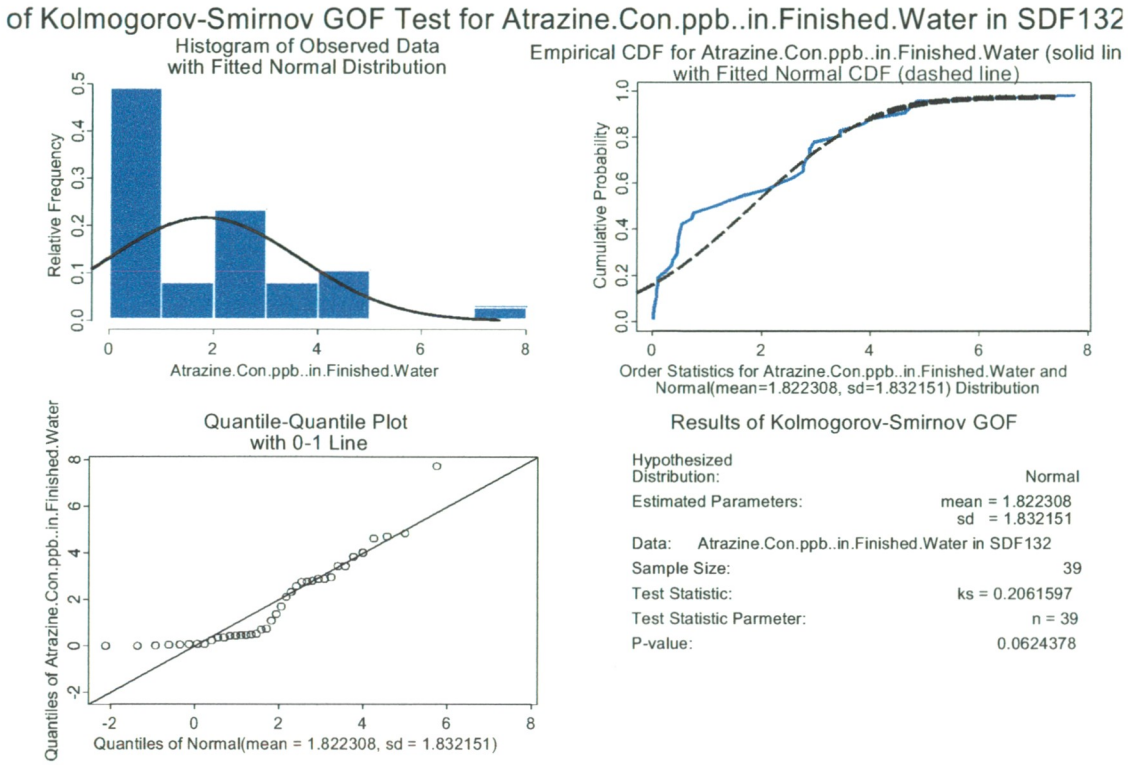


Results of Kolmogorov-Smirnov GOF

Hypothesized Distribution:	Normal
Estimated Parameters:	mean = 0.5058621 sd = 0.5468515
Data:	Atrazine.Conc.ppb..in.Finished.Water in SDF131
Sample Size:	29
Test Statistic:	ks = 0.3318627
Test Statistic Parmeter:	n = 29
P-value:	0.002389359

```
Results of Goodness-of-Fit Test
-------------------------------
Test Method:                     Kolmogorov-Smirnov GOF

Hypothesized Distribution:       Normal

Estimated Parameter(s):          mean = 0.5058621
                                 sd   = 0.5468515

Estimation Method:

Data:                            Atrazine.Conc.ppb...In.Finished.Water in SDF131

Number NA/NaN/Inf's Removed:     3

Sample Size:                     29

Test Statistic:                  ks = 0.3318627

Test Statistic Parameter:        n = 29

P-value:                         0.002389359

Alternative Hypothesis:          True cdf does not equal the
                                 Normal Distribution.
```

Figure 8: Kolmogorov-Smirnov goodness-of-fit test for the Study Period 2002




```
 Results of Goodness-of-Fit Test
-------------------------------

Test Method:                    Kolmogorov-Smirnov GOF

Hypothesized Distribution:      Normal

Estimated Parameter(s):         mean = 1.822308
                                sd   = 1.832151

Estimation Method:

Data:                           Atrazine.Con.ppb...In.Finished.Water in SDF132

Sample Size:                    39

Test Statistic:                 ks = 0.2061597

Test Statistic Parameter:       n = 39

P-value:                        0.0624378

Alternative Hypothesis:         True cdf does not equal the
                                Normal Distribution.
```

Quarterly Analysis of Data

The concentrations of atrazine in finished water, projected as sets of quarterly samples for the years 2000, 2001, and 2002 are presented in Figures 9, 10, and 11. Data were sampled according to the previous methodology and are representative of the first collected sample for each month sub-sampled for the three years 2000, 2001 and 2001, respectively. These samples can be considered to be collected in the first week of the month sub-sampled in a particular year. Additionally, the analysis was expanded to assess other patterns, such as the third week of the month, to address the most appropriate quarterly sampling regime (Figures 12, 13, and 14).

Figure 9: Atrazine Concentration in Quarterly Samples of Finished Drinking Water at Lewisburg Water Treatment Plant, 2000

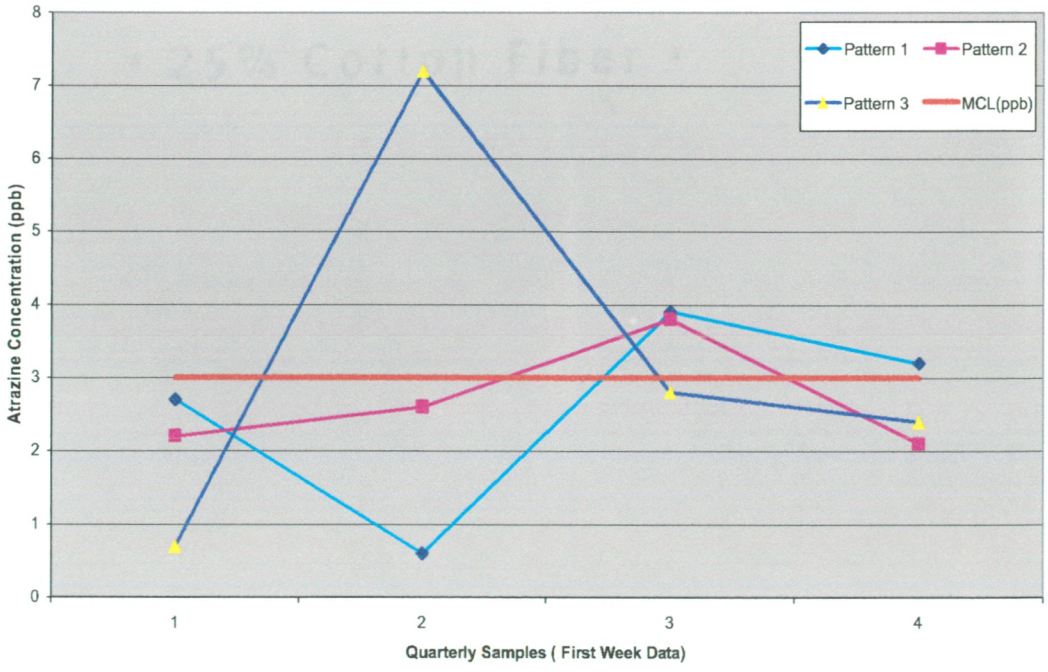


Figure 10: Atrazine Concentration in Quarterly Samples of Finished Drinking Water at Lewisburg Water Treatment Plant, 2001

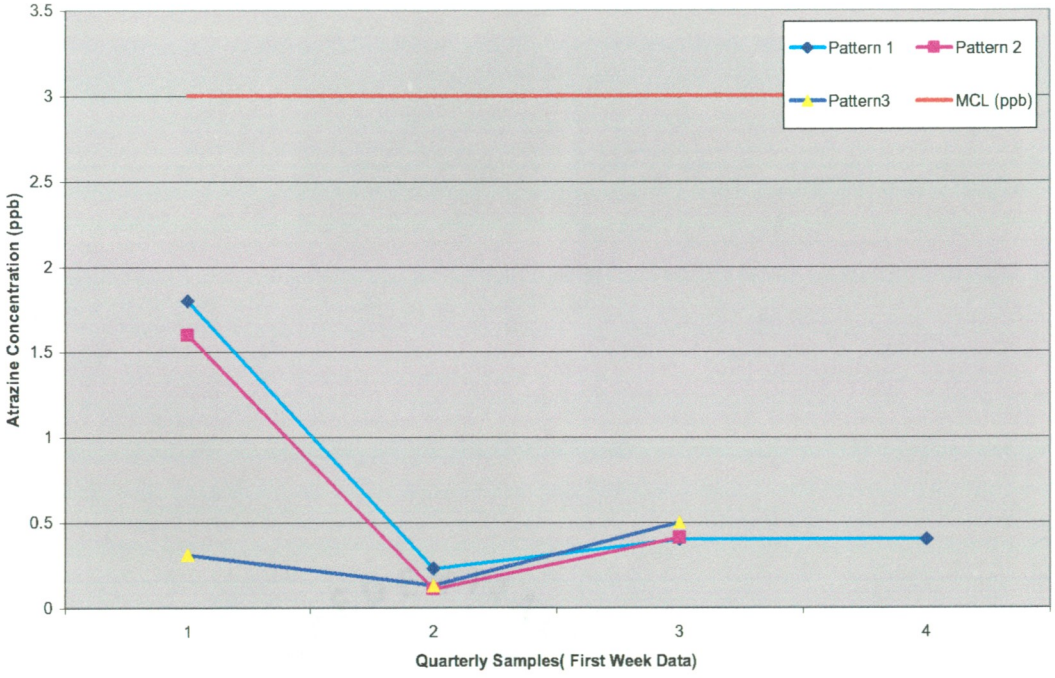


Figure 11: Atrazine Concentration in Quarterly Samples of Finished Drinking Water at Lewisburg Water Treatment Plant, 2002

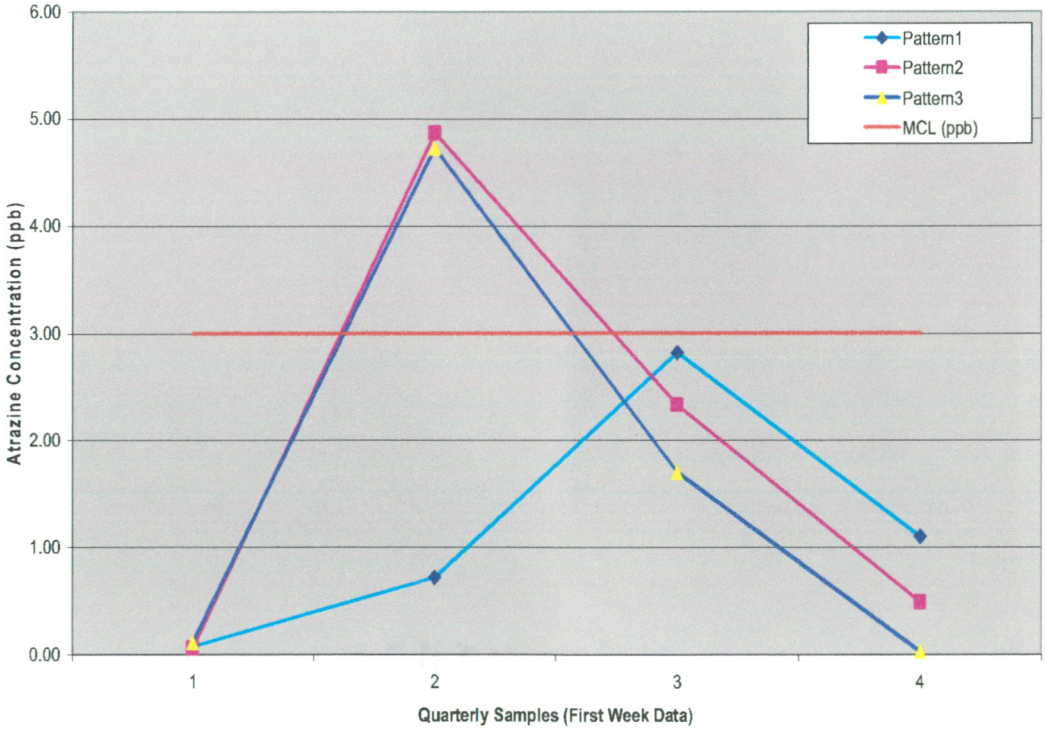


Figure 12: Atrazine Concentration in Quarterly Samples of Finished Drinking Water at Lewisburg Water Treatment Plant, 2000

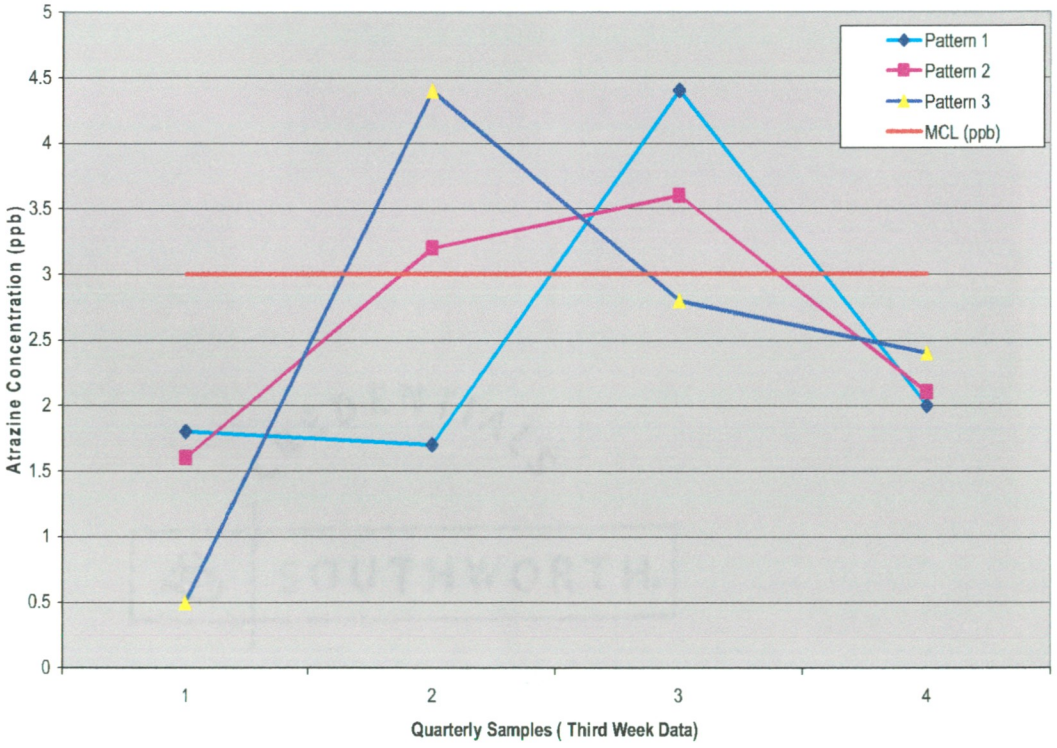


Figure 13: Atrazine Concentration in Quarterly Samples of Finished Drinking Water at Lewisburg Water Treatment Plant, 2001

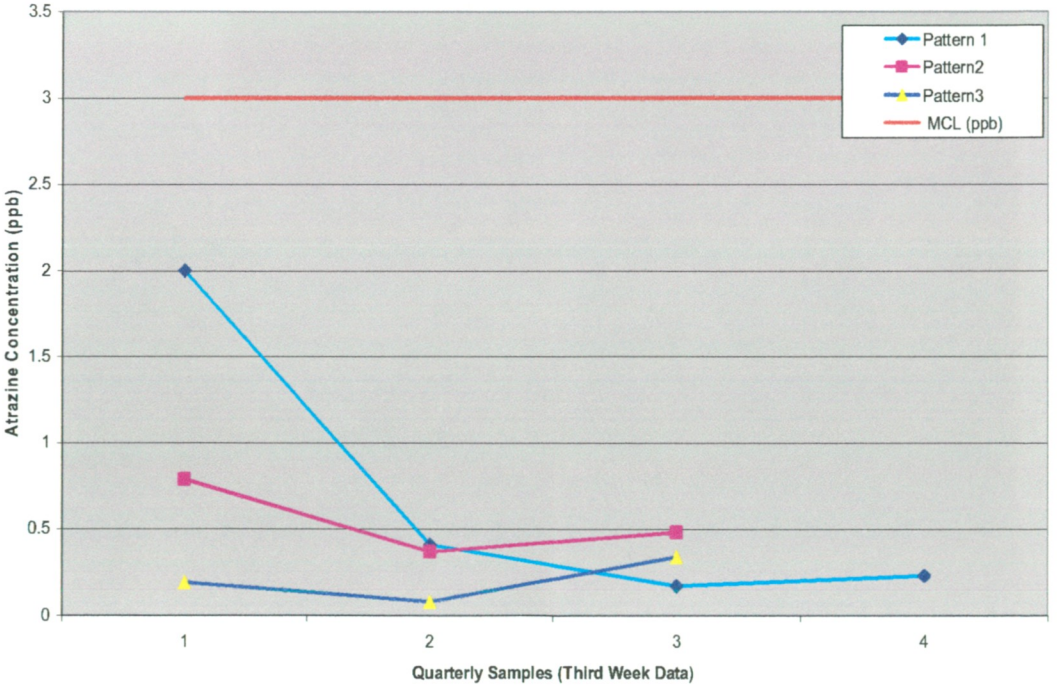
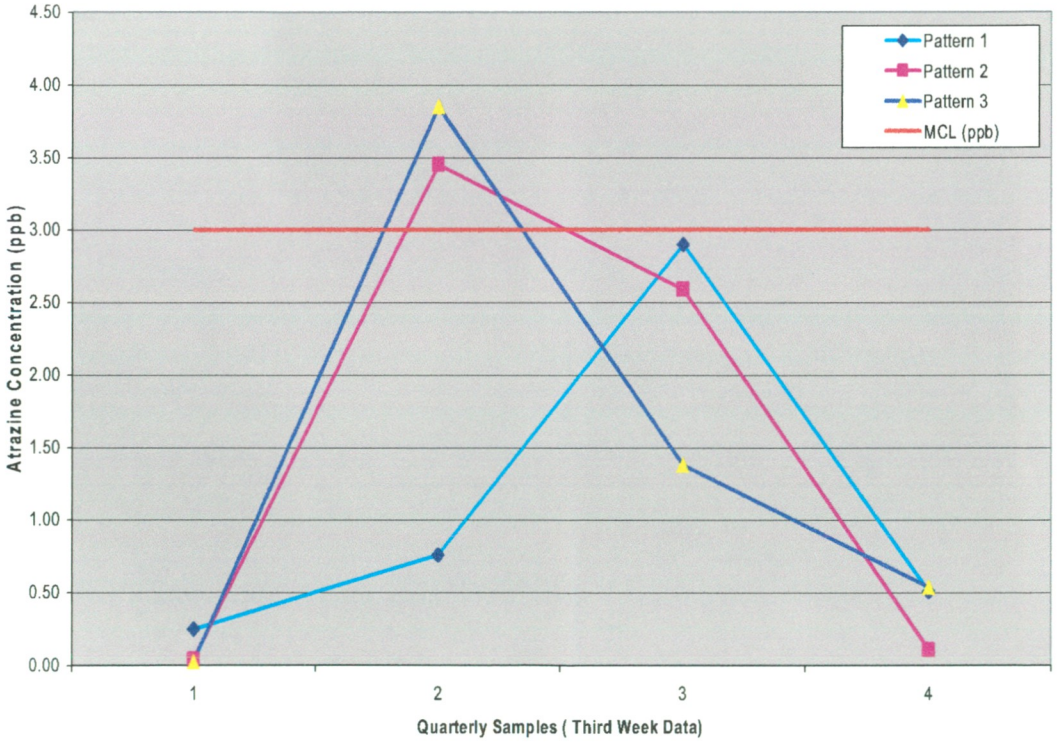


Figure 14: Atrazine Concentration in Quarterly Samples of Finished Drinking Water at Lewisburg Water Treatment Plant, 2002



For comparison, data for the maximum recorded concentrations obtained from each month's sampling data for the study periods 2000, 2001, and 2002 are graphically represented in Figures 15, 16, and 17, respectively. The maximum concentrations were used in the quarterly sampling strategy and applied to each of the three patterns for each study period.

The comparison of the mean values between each pattern for every study period (year 2000, 2001, and 2002) and also with the annual mean of all the samples for a particular year and the annual mean of the first week, the third week, and the highest concentration samples is shown in the Table 14. All the values are represented in ppb (parts per billion).

Figure 15: Maximum Recorded Atrazine Concentrations in Finished Drinking Water at Lewisburg Water Treatment Plant, 2000

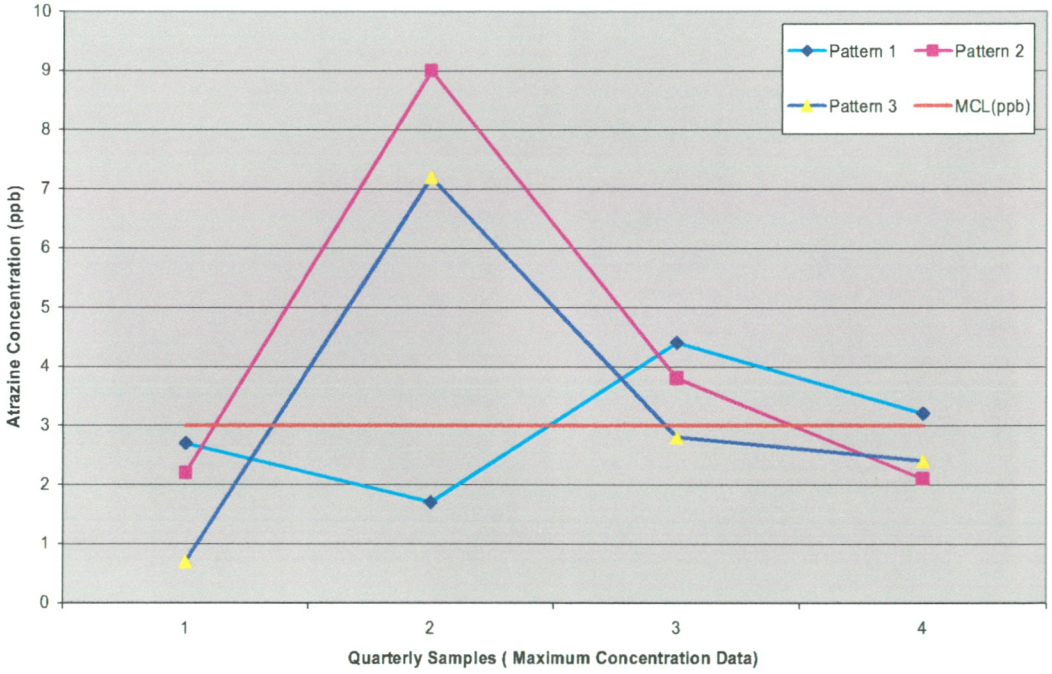


Figure 16: Maximum Recorded Atrazine Concentration in Finished Drinking Water at Lewisburg Water Treatment Plant, 2001

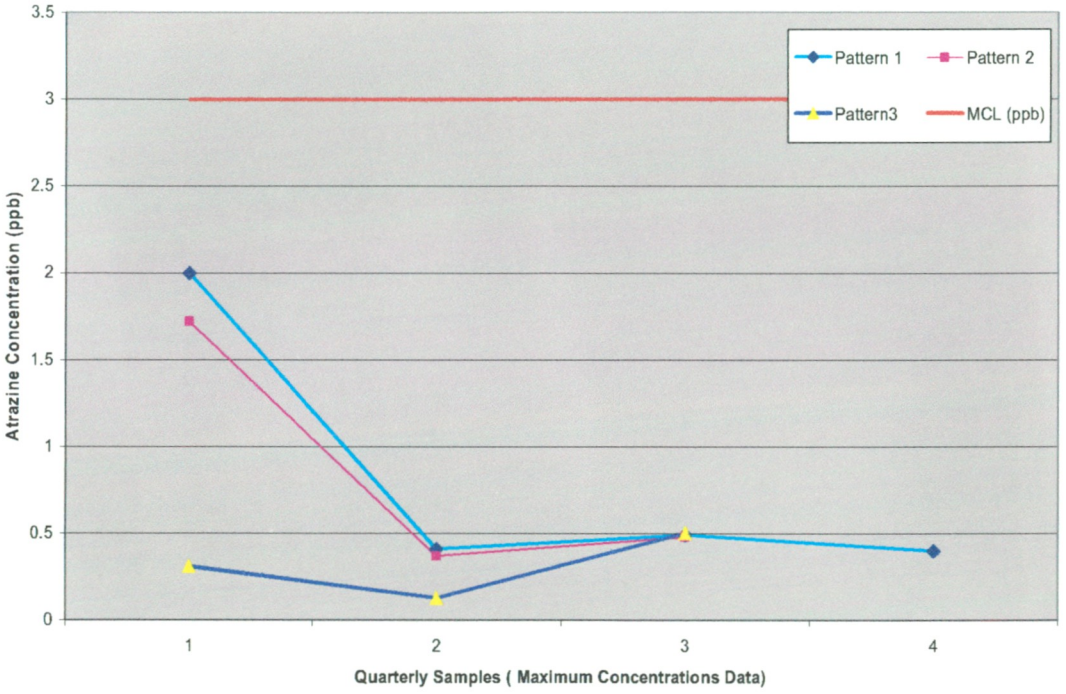
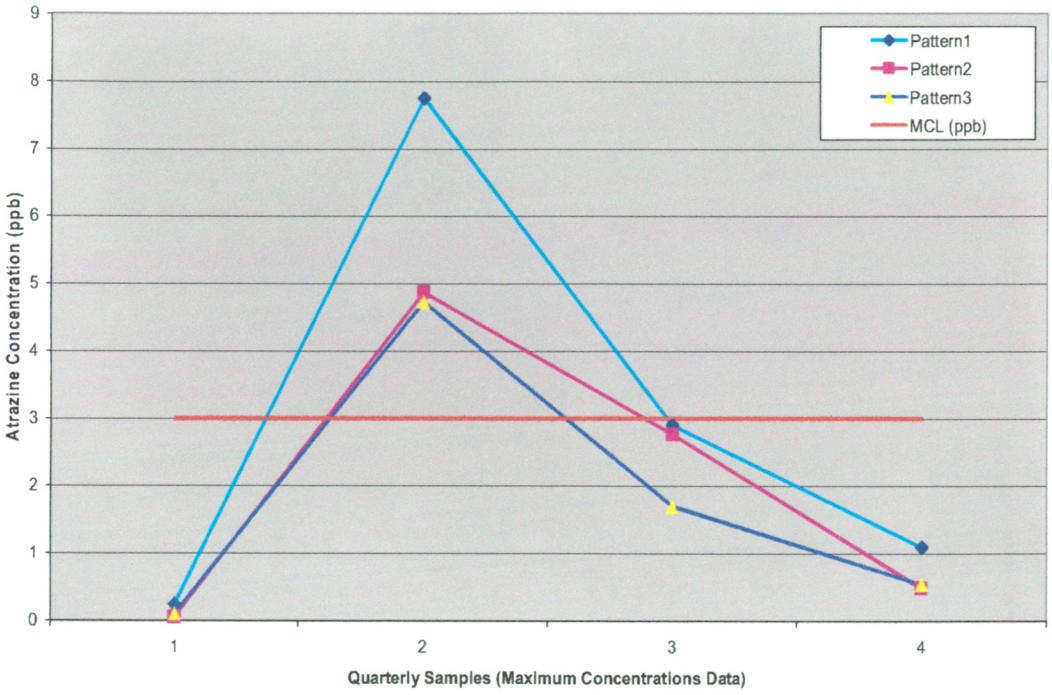


Figure 17: Maximum Recorded Atrazine Concentration in Finished Drinking Water at Lewisburg Water Treatment Plant, 2002



Chapter-5

DISCUSSION

A discussion of the results is presented in this section. Included within the discussion are a comparison of the annual trend concentration data, comparison of quarterly sub-samples, and a discussion of the relevance to drinking water systems.

Distribution and Occurrence of Atrazine (use same titles as in Results)

The data from the first study period (year 2000) was subjected to the goodness-of-fit test. Data from 2000 was found to be normally distributed, as evidenced by the graphical analysis. The Null Hypothesis for the test was that the data are normally distributed (p value > 0.05). According to the to the KS goodness-of-fit test, the p value for the first study period was calculated to be greater than 0.05 (p=0.306). This test indicates the validity of Null Hypothesis for this dataset, that the Null should be accepted. Likewise the data from the second study period (year 2001) and the third study period (year 2002) was subjected to the goodness-of-fit tests. Their p values were (p=0.0023) and (p=0.062), respectively. The data from the second study period was not normally distributed (since p value<0.05), and the Null Hypothesis was rejected. It was known from the weather databases that a drought occurred in 2001, which resulted in low values in the concentration of atrazine in the raw and finished waters. It can be inferred from this observation that the non-normal distribution of the data during this time period can be attributed to the seasonal variation caused by a

drought in the region around the Spa Lake watershed. The data in the third study period was determined to be normally distributed (since p value > 0.05). This statistical analysis summarizes the goodness-of-fit tests performed on the three datasets. The first and the third study periods were normally distributed (years 2000 and 2002), whereas the second study period (year 2001) was not normally distributed.

The technical fact sheet on atrazine, as published by the U.S Environmental Protection Agency for Ground Water and Drinking Water, set forth the regulatory protocol to be followed by all drinking water systems for the treatment of atrazine in drinking water. The initial monitoring frequency suggests the collection and analysis of four quarterly samples every three years. With the results from the goodness-of-fit tests for the three study periods, there would be a possibility that the sampling protocol could be misleading due to the presence of extremely low concentrations of atrazine in finished drinking water in the drought year and the occurrence of a non-normal distribution (year 2001). This variation is a point of concern because the monitoring strategy insists on returning to the initial frequency of sampling if detection limit is greater than 1 ppb. The mean of all the samples collected and analyzed for the concentration of atrazine in finished water for the first study period (year 2000) is 3.06 ppb (Table 14). This value is above the MCL as set by the Safe Drinking Water Act. Also, it is well above the 1 ppb, which would result in a repeat of the initial frequency of sampling for atrazine in finished water at the water treatment plant. The calculated mean for all the samples of finished water for the second (year 2001) and third study periods (year 2002) is 0.5 ppb and 1.82 ppb,

respectively. Results show that if initial sampling at the water treatment plant was to start in the year 2001, it could result in the conclusion that the atrazine levels in finished water is below 1 ppb as set by the U.S.E.P.A for a return to initial frequency of sampling. Also it can be noticed that the mean is well below the MCL for atrazine, which is 3 ppb. But, if the initial sampling of the water treatment plant were to begin in either 2000 or 2002, the mean concentration of atrazine in finished drinking water at the water treatment plant is indicative of the high values. The mean of 1.82 ppb for the concentration of atrazine in finished water at the LWTP for the year 2002 exceeds the 1 ppb limit resulting in the repeat sampling of the water treatment plant using the quarterly sampling strategy. These means explain the importance of observing the variation and distribution of atrazine concentrations over a series of years, rather than relying on a single year of data or a single quarter of data.

Comparison of Quarterly Trends

The results from the analysis of the data for the three quarterly sampling patterns of each year are discussed here. In the year 2000, the data from the analysis of the concentration of atrazine in finished water collected in the first week of every month were studied. The mean value of the four atrazine finished water samples was calculated to be 2.6 ppb. This value was compared to the mean from pattern 2, and 3 which led to the conclusion that the mean or average of the concentration of atrazine in finished water samples was not identical. The mean concentrations of the data from patterns 1 and 2 were 2.6 ppb, which was below the MCL; whereas in pattern 3 the recorded mean concentration in finished water was 3.275, greater than the MCL.

These results display a disparity in terms of the allowed concentrations of atrazine in drinking water as two patterns are below the MCL, and one is above the MCL. From this comparison to the MCL, it is quite obvious that regulatory concentrations vary by the pattern selected for sampling for a particular year and could result in the application or non-application of the regulatory measures at the water treatment plant. Likewise the atrazine concentrations for finished water for the three patterns for each year were compared during the years 2001 and 2002(Table 14). According to this comparison, it was observed that the mean concentrations of atrazine for the three patterns in the three years varied from the MCL with recorded values both above and below the MCL. The highest concentrations of atrazine in finished water during each month were studied to determine the difference between the observations made by the normal pattern of sampling and the true concentrations present in the finished water throughout a period of one year. This study was done because it was noticed that some of the high concentrations of atrazine were missed in the first and third week sampling strategy. For the year 2000, the mean calculated for pattern 2 using the first sample of the month was 2.67 ppb; whereas for the same pattern and for the same year using the highest concentration values during that pattern resulted in a value of 4.27 ppb, which is significantly above the MCL. This result exposes the chance of missing the high concentration of atrazine by the use of a particular pattern of sampling, thereby resulting in the continued exposure to high concentrations of atrazine in finished drinking water, without the knowledge of facility personnel or the public. This type of variation was also observed for the first pattern in the year 2002 for the mean values calculated using the first and third samples of a month and the

highest concentration values of the same month. This analysis explains the presence of concentration of atrazine at or above the MCL during a particular year, which could be missed in the sampling pattern or strategy employed.

Comparison of Annual and Quarterly Data

The annual mean using all the samples collected in a particular year was compared with the annual mean obtained using the quarterly sub-sample of the first sample of each month (Table 14). Though the number of observations (n) for both the datasets is different, the possibility of a sampling strategy of collecting one sample a month or collecting a water sample a week does exist. This study shows the comparison of the possibilities of the two sampling methodologies, and the variation that would exist if these sampling strategies were in effect. It was observed that for the year 2000, the annual mean for all the samples (3.06 ppb) exceeded the MCL (3 ppb); whereas the annual mean calculated using the quarterly method of the first sample of each month (2.85) was found to be below the MCL (3ppb). This comparison has regulatory significance as the two values lie on either side of the MCL, thus one value is in violation and the other is not. The inference is that there could be a possibility of missing some of the high concentrations of atrazine in water especially during the application season, when a quarterly sampling strategy is used. The same trend was also observed with the annual mean of all the samples collected in the year 2000 (3.06 ppb) exceeding the MCL (3 ppb) when the annual mean of the third sample of every month for the same year (2.54 ppb) was below the MCL (3 ppb). When the annual mean using the quarterly sub-sample of the first sample and

the third sample of each month for a year was compared with that of the highest concentration sample of each month, it was observed that the mean obtained from the data representing the highest concentration of each month was higher than the mean obtained from the first and third samples of each month for all the three years (n=12 for each year). This variation could support the possibility of the concentration of atrazine to be high and yet be missed by following a particular sampling pattern or strategy. This discussion supports the research objective that the current methodology for small water systems to test for atrazine contamination in finished drinking water on a quarterly basis does not provide an accurate representation of the annual variation in the distribution of atrazine. This lack of accuracy in representation is due to the variation observed in the concentrations of the atrazine in finished water for various combinations of patterns of sampling data with reference to the annual mean calculated using the whole year's dataset. This discussion supports the alternative hypothesis that there is a significant regulatory difference between the quarterly sampling strategy and the annual atrazine data distribution in finished drinking water for small water systems experiencing atrazine contamination problems.

Chapter-6

CONCLUSION

The results of this study led to an increased understanding of the distribution and variation in the concentration of atrazine in finished drinking water at the LWTP. One of the major limitations of this study is that it was a purposive study and sampling was not randomly selected. The other limitation, which could be significant, is the occasional lapse in the data due to irregularities in sampling and transport. The use of the technique of immunoassay for the analysis of the samples is to be noted; however, this method was cost effective and provided the necessary resolution in the data. Analysis of the data led to new questions to be answered about the distribution and occurrence of atrazine:

1. Is there an adequate protocol for the sampling of raw and finished water at community water systems suspected of having atrazine contamination to express the annual distribution and variation?
2. Is there a methodology to determine the time of the initiation of the quarterly sampling procedure at a water system that has atrazine contamination?

This study created new challenges in the study of the distribution of atrazine in an agricultural watershed, and the patterns of its variation in terms of climate and environmental fate are to be further studied and analyzed. The high concentrations of this herbicide during different times of the year could lead to a difference in the

exposure levels through drinking water. This field of study is one that can be expanded based on the awareness of the wide usage of atrazine leading to contamination of source waters in community water systems located in agricultural watersheds. The transport of atrazine to streams, rivers, groundwater, and reservoirs occurs primarily by nonpoint source runoff from fields, which needs to be assessed with more specificity to understand the environmental transport and transformation of atrazine. This could in turn affect atrazine concentrations in raw and finished waters.

The involvement of stakeholders in the issue of source water impairment by atrazine contamination would be a major step to the reduction of the concentrations of atrazine in drinking water. This need calls for awareness and education programs not only among the farming community but also the general public with an increased encouragement for the implementation of Best Management Practices (BMPs). The demonstrated program of immunoassay assessment of a community water system was an effective means to generate information for public education and relay that information to the farming, regulatory, and water communities. In this manner, scientific data was used to increase the likelihood of stakeholder input and action, as the farmers participated in an atrazine reduction incentive program. Also, the collection of local data on contamination problems increased the probability of financial assistance to install BMPs, resulting in the farmers in the Spa Lake basin receiving incentive funds.

This study illustrates the need to further study the basin and field characteristics which may be important factors controlling atrazine levels in the water supply reservoir. There is a need for a long-term monitoring program to be continued,

coupled with meteorological sampling, and land use analysis in order to quantify the trend in atrazine concentrations following sustained reductions in use. This study could be utilized for the assessment of the environmental occurrence and distribution of atrazine as a tool for public health and source water awareness in other agricultural watersheds. Through an integrated approach involving the local stakeholders, the consumers and the bureaucracy, an effective program should be supported by monitoring to assess the implementation of BMPs. Results of this program showed monitoring increased participation and provided for sustenance and feedback to a network for herbicide/pesticide management decisions statewide, the Kentucky Pesticide Workgroup. Ultimately, this research can serve as a model for other agricultural watersheds with similar drinking water concerns and provide for a baseline to protect public health from atrazine contamination.

Glossary

U.S EPA: United States Environmental Protection Agency.

MCL: Maximum Contaminant Level.

MCLG: Maximum Contaminant Level Goals

SDWA: Surface Drinking Water Act.

ELISA: Enzyme Linked Immuno Sorbent Assay.

Gas Chromatography (GC): Involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

Source Water: Source water is untreated water from streams, rivers, lakes, or underground aquifers which is used to supply private wells and public drinking water. Most public and some private well drinking waters are treated before it enters our homes. While some treatment is usually necessary, the costs of treatment and risks to public health can be reduced by ensuring that source water is protected from contamination.

Source Water Protection: Protection of drinking water at the source can be successful in providing public health protection and reducing the treatment challenge for public water suppliers. Source water quality can be threatened by many everyday activities and land uses, ranging from industrial wastes to the chemicals applied to suburban lawns.

APPENDICES

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APPENDIX--A

Table 1 represents the recorded data from May 1999 to April 2000.

Table 1: Atrazine analysis for the Year May 1999 to April 2000

Atrazine analysis for the Year May 1999- April 2000	Date	Raw Water	Finished Water
Analysis Method Type	**Sample Date**	**Atrazine Conc.(ppb)**	**Atrazine Conc.(ppb)**
Immunoassay	5/10/1999	17	14
Immunoassay	5/17/1999	12	12
Immunoassay	5/24/1999	14	12
Immunoassay	6/1/1999	12	11
Immunoassay	6/7/1999	5.5	8.5
Immunoassay	6/14/1999	5	4.8
Immunoassay	6/21/1999	6.5	6
Immunoassay	6/28/1999	4	4
Immunoassay	7/6/1999	1.5	1.3
Immunoassay	7/12/1999	3.6	3.1
Immunoassay	7/19/1999	3.1	2.5
Immunoassay	7/26/1999	2.6	2
Immunoassay	8/2/1999	3.2	2.3
Immunoassay	9/13/1999	3.2	2.5
Immunoassay	9/20/1999	2.7	2.2
Immunoassay	10/4/1999	2.6	2.2

Immunoassay	10/19/1999	3.4	2.7
Immunoassay	11/3/1999	4.2	2.7
Immunoassay	11/15/1999	3	2.4
Immunoassay	11/29/1999	3.8	3.1
Immunoassay	12/13/1999	2.1	2.1
Immunoassay	1/3/2000	3.1	2.7
Immunoassay	1/24/2000	3.2	1.8
Immunoassay	2/7/2000	2.6	2.2
Immunoassay	2/21/2000	1.7	1.6
Immunoassay	3/6/2000	0.9	0.7
Immunoassay	3/20/2000	0.8	0.5
Immunoassay	4/3/2000	1	0.6
Immunoassay	4/17/2000	1.7	1.7

Table 2 represents the recorded data from May 2000 to April 2001.

Table 2: Atrazine analysis for the Year May 2000 to April 2001

Atrazine analysis for the Year 2000	Date	Raw Water	Finished Water
Analysis Method Type	**Sample Date**	**Atrazine Conc.(ppb)**	**Atrazine Conc.(ppb)**
Immunoassay	5/1/2000	2.7	2.6
Immunoassay	5/8/2000	2.8	2.7
Immunoassay	5/15/2000	3.3	2.4
Immunoassay	5/22/2000	3.2	3.2
Immunoassay	5/30/2000	6.5	9
Immunoassay	6/5/2000	3.6	7.2
Immunoassay	6/12/2000	5.2	6.4
Immunoassay	6/19/2000	3.2	4.4
Immunoassay	6/26/2000	3.6	3.8
Immunoassay	7/10/2000	3.3	3.9
Immunoassay	7/17/2000	4.6	4.4
Immunoassay	7/24/2000	3.8	3
Immunoassay	8/14/2000	4.4	3.8
Immunoassay	8/21/2000	4.4	3.6
Immunoassay	9/5/2000	3.4	2.8
Immunoassay	9/18/2000	3	2.8
Immunoassay	10/4/2000	3.6	3.2
Immunoassay	10/16/2000	2.7	2
Immunoassay	10/30/2000	2.8	2.4
Immunoassay	11/13/2000	2.2	2.1
Immunoassay	11/27/2000	2.8	2.1
Immunoassay	12/11/2000	2.7	2.4
Immunoassay	1/8/2001	2	1.8
Immunoassay	1/22/2001	2.4	2

Immunoassay	2/5/2001	2.2	1.6
Immunoassay	2/15/2001	3.40	1.72
Immunoassay	2/26/2001	0.81	0.79
Immunoassay	3/12/2001	0.36	0.31
Immunoassay	3/26/2001	0.27	0.19
Immunoassay	4/9/2001	0.26	0.23
Immunoassay	4/23/2001	0.47	0.41

Table 3 represents the recorded data from May 2001 to April 2002.

Table 3: Atrazine analysis for the Year May 2001 to April 2002

Atrazine analysis for the Year 2001	Date	Raw Water	Finished Water
Analysis Method Type	**Sample Date**	**Atrazine Conc.(ppb)**	**Atrazine Conc.(ppb)**
Immunoassay	5/7/2001	0.11	0.11
Immunoassay	5/14/2001	0.19	0.17
Immunoassay	5/21/2001	0.53	0.37
Immunoassay	5/29/2001	0.12	0.20
Immunoassay	6/4/2001	0.25	0.13
Immunoassay	6/11/2001	0.11	0.05
Immunoassay	6/18/2001	0.24	0.04
Immunoassay	6/25/2001	0.24	0.08
Immunoassay	7/2/2001	0.59	0.40
Immunoassay	7/9/2001	0.59	0.48
Immunoassay	7/17/2001	0.63	0.49
Immunoassay	7/23/2001	0.27	0.17
Immunoassay	7/30/2001	0.34	0.20
Immunoassay	8/6/2001	0.52	0.41
Immunoassay	8/20/2001	0.67	0.48
Immunoassay	9/4/2001	0.51	0.50
Immunoassay	9/17/2001	0.52	0.34
Immunoassay	10/1/2001	0.55	0.40
Immunoassay	10/15/2001	0.52	0.37
Immunoassay	10/29/2001	0.42	0.23
Immunoassay	11/12/2001	NA	NA
Immunoassay	11/26/2001	NA	NA
Immunoassay	12/10/2001	NA	NA
Immunoassay	1/7/2002	0.20	0.08

Immunoassay	1/22/2002	0.37	0.25
Immunoassay	2/4/2002	0.09	0.06
Immunoassay	2/18/2002	0.07	0.04 nd
Immunoassay	3/4/2002	0.07	0.11
Immunoassay	3/18/2002	0.03 nd	0.03 nd
Immunoassay	4/4/2002	1.53	0.72
Immunoassay	4/15/2002	1.28	0.76
Immunoassay	4/29/2002	13.35	7.75

Table 4 represents the recorded data from May 2002 to April 2003.

Table 4: Atrazine analysis for the Year May 2002 to April 2003

Atrazine analysis for the Year 2002 **Analysis Method Type**	Date **Sample Date**	Raw Water **Atrazine Conc.(ppb)**	Finished Water **Atrazine Conc.(ppb)**
Immunoassay	5/13/2002	* 5.74	4.87
Immunoassay	5/20/2002	* 5.57	3.45
Immunoassay	5/28/2002	* 5.92	4.64
Immunoassay	6/3/2002	4.90	4.73
Immunoassay	6/10/2002	4.22	4.03
Immunoassay	6/17/2002	4.25	3.85
Immunoassay	6/24/2002	3.69	3.45
Immunoassay	7/1/2002	2.58	2.82
Immunoassay	7/8/2002	2.94	2.78
Immunoassay	7/15/2002	2.85	2.90
Immunoassay	7/22/2002	2.74	2.90
Immunoassay	7/29/2002	3.09	2.96
Immunoassay	8/5/2002	* 6.00	2.33
Immunoassay	8/12/2002	2.43	2.77
Immunoassay	8/19/2002	2.77	2.59
Immunoassay	8/26/2002	3.16	2.13
Immunoassay	9/9/2002	2.91	1.70
Immunoassay	9/23/2002	2.81	1.38
Immunoassay	9/30/2002	**	0.45
Immunoassay	10/7/2002	**	1.10
Immunoassay	10/14/2002	0.70	0.46
Immunoassay	10/21/2002	0.74	0.51
Immunoassay	10/28/2002	0.83	0.47
Immunoassay	11/4/2002	0.85	0.49
Immunoassay	11/11/2002	0.60	0.38

Immunoassay	11/25/2002	0.16	0.11
Immunoassay	12/2/2002	0.20	0.03
Immunoassay	12/9/2002	0.38	0.36
Immunoassay	12/16/2002	0.27	0.54
Immunoassay	12/30/2002	0.12	0.09
Immunoassay	1/6/2003	0.18	0.13
Immunoassay	1/13/20003	0.17	0.11
Immunoassay	1/20/2003	0.09	0.02
Immunoassay	1/27/2003	0.07	0.04
Immunoassay	2/3/2003	0.04	0.04
Immunoassay	2/10/2003	0.04	0.04
Immunoassay	2/17/2003	**	0.07
Immunoassay	2/24/2003	**	0.08
Immunoassay	3/3/2003	0.1	0.06
Immunoassay	3/10/2003	0.07	0.12
Immunoassay	3/17/2003	0.13	0.13
Immunoassay	3/24/2003	0.14	0.12
Immunoassay	3/31/2003	0.31	0.2
Immunoassay	4/7/2003	0.75	0.62

Foot Notes: 1.nd - non-detect

2. ** - Sample bottles broken upon arrival at the lab

3. NA- Data Not Available

4. * - above detection limit

The available data is arranged into three year study periods. Each study period ranges from the month of January to December. The study periods are designed for the three years 2000, 2001, and 2002. The data from the study period 2000 was represented as a graph which shows the variation in the concentration of atrazine in both the raw and finished water during the year 2000 (Graph 2). The presence of raw

water concentrations in the graphical representation of this study period signifies the comparative analysis to be undertaken to identify the trends in the distribution of atrazine in finished drinking water over a period of one year.

The following three tables are representative of the three study periods.

Table 5: Study Period 2000

Year 2000

Sample Date	Atrazine Conc.(ppb) in Raw Water	Atrazine Conc.(ppb) in Finished Water
1/3/2000	3.1	2.7
1/24/2000	3.2	1.8
2/7/2000	2.6	2.2
2/21/2000	1.7	1.6
3/6/2000	0.9	0.7
3/20/2000	0.8	0.5
4/3/2000	1	0.6
4/17/2000	1.7	1.7
5/1/2000	2.7	2.6
5/8/2000	2.8	2.7
5/15/2000	3.3	2.4
5/22/2000	3.2	3.2
5/30/2000	6.5	9
6/5/2000	3.6	7.2
6/12/2000	5.2	6.4
6/19/2000	3.2	4.4
6/26/2000	3.6	3.8
7/10/2000	3.3	3.9
7/17/2000	4.6	4.4
7/24/2000	3.8	3
8/14/2000	4.4	3.8
8/21/2000	4.4	3.6
9/5/2000	3.4	2.8
9/18/2000	3	2.8
10/4/2000	3.6	3.2
10/16/2000	2.7	2

10/30/2000	2.8	2.4
11/13/2000	2.2	2.1
11/27/2000	2.8	2.1
12/11/2000	2.7	2.4

The data from the study period 2001 was represented as a graph which shows the variation in the concentration of atrazine in both the raw and finished water during the year 2001 (Graph 3).

Table 6: Study Period 2001

Year 2001

Sample Date	Atrazine Conc.(ppb) in Raw Water	Atrazine Conc.(ppb) in Finished Water
1/8/2001	2	1.8
1/22/2001	2.4	2
2/5/2001	2.2	1.6
2/15/2001	3.40	1.72
2/26/2001	0.81	0.79
3/12/2001	0.36	0.31
3/26/2001	0.27	0.19
4/9/2001	0.26	0.23
4/23/2001	0.47	0.41
5/7/2001	0.11	0.11
5/14/2001	0.19	0.17
5/21/2001	0.53	0.37
5/29/2001	0.12	0.20

6/4/2001	0.25	0.13
6/11/2001	0.11	0.05
6/18/2001	0.24	0.04
6/25/2001	0.24	0.08
7/2/2001	0.59	0.40
7/9/2001	0.59	0.48
7/17/2001	0.63	0.49
7/23/2001	0.27	0.17
7/30/2001	0.34	0.20
8/6/2001	0.52	0.41
8/20/2001	0.67	0.48
9/4/2001	0.51	0.50
9/17/2001	0.52	0.34
10/1/2001	0.55	0.40
10/15/2001	0.52	0.37
10/29/2001	0.42	0.23
11/12/2001	NA	NA
11/26/2001	NA	NA
12/10/2001	NA	NA

The data from the study period 2002 was represented as a graph which shows the variation in the concentration of atrazine in both the raw and finished water during the year 2002 (Graph 4).

Table 7: Study Period 2002

Year 2002

Sample Date	Atrazine Conc.(ppb) in Raw Water	Atrazine Conc.(ppb) in Finished Water
1/7/2002	0.20	0.08
1/22/2002	0.37	0.25
2/4/2002	0.09	0.06
2/18/2002	0.07	0.04
3/4/2002	0.07	0.11
3/18/2002	0.03	0.03
4/4/2002	1.53	0.72
4/15/2002	1.28	0.76
4/29/2002	13.35	7.75
5/13/2002	5.74	4.87
5/20/2002	5.57	3.45
5/28/2002	5.92	4.64
6/3/2002	4.90	4.73
6/10/2002	4.22	4.03
6/17/2002	4.25	3.85
6/24/2002	3.69	3.45
7/1/2002	2.58	2.82
7/8/2002	2.94	2.78
7/15/2002	2.85	2.90
7/22/2002	2.74	2.90
7/29/2002	3.09	2.96
8/5/2002	6.00	2.33
8/12/2002	2.43	2.77
8/19/2002	2.77	2.59
8/26/2002	3.16	2.13
9/9/2002	2.91	1.70
9/23/2002	2.81	1.38

9/30/2002	**	0.45
10/7/2002	**	1.10
10/14/2002	0.70	0.46
10/21/2002	0.74	0.51
10/28/2002	0.83	0.47
11/4/2002	0.85	0.49
11/11/2002	0.60	0.38
11/25/2002	0.16	0.11
12/2/2002	0.20	0.03
12/9/2002	0.38	0.36
12/16/2002	0.27	0.54
12/30/2002	0.12	0.09

Foot Notes: 1.nd - non-detect

2. ** - Sample bottles broken upon arrival at the lab

3. NA- Data Not Available

4. * - above detection limit

The data for each study period (years 2000, 2001, &2002) are analyzed using summary statistics to observe the variation in the mean for the entire data set. This is recorded below in Table 14.

Table 14:

Using First Week Samples					
Year	**Mean Value for Pattern 1**	**Mean Value for Pattern 2**	**Mean Value for Pattern 3**	**Annual Mean using all samples**	**Annual Mean using the first sample of each month**
2000	2.6	2.67	3.275	3.06	2.85
2001	0.7	0.7	0.31	0.5	0.58
2002	1.18	1.93	1.64	1.82	1.58
Using Third Week Samples					
Year	**Mean Value for Pattern 1**	**Mean Value for Pattern 2**	**Mean Value for Pattern 3**	**Annual Mean using all samples**	**Annual Mean using the third sample of each month**
2000	2.47	2.62	2.52	3.06	2.54
2001	0.7	0.54	0.2	0.5	0.5
2002	1.1	1.54	1.45	1.82	1.36
Using the Highest Conc. values of each month					
Year	**Mean Value for Pattern 1**	**Mean Value for Pattern 2**	**Mean Value for Pattern 3**	**Annual Mean using all samples**	**Annual Mean using the highest Conc. sample of each month**
2000	3	4.27	3.27	3.06	3.51
2001	0.825	0.85	0.31	0.5	0.68
2002	3.01	2.04	1.77	1.82	2.27

APPENDIX--B

Quarterly Analysis

The following set of tables represents the concentrations of atrazine in raw and finished water projected as sets of quarterly samples for the years 2000, 2001, and 2002. The current methodology which calls for quarterly sampling in a year is taken as the basis for this strategy in statistical analysis and representation.

Put this methodology description in the methodology section in results you just present the results

The quarterly sampling strategy could be devised for each year by dividing it into equal sampling intervals in terms of time duration between the collection of the first sample and the next. Each year was divided into sampling patterns. Three patterns for quarterly sampling are identified in each year. The first pattern represents the months January, April, July, and October. The second pattern represents the months February, May, August, and November. The third pattern represents the months March, June, September, and December.

The concentrations of atrazine for raw and finished water in this set of tables (8, 9, and 10) are representative of the first collected sample for each month for the three years. These samples can be considered to be collected in the first week of every month in a particular year.

Table 8 represents the data recorded for the three patterns in the year 2000.

Table 8:

For the Year 2000				
Pattern	**Month**	**Sample Date**	**Atrazine Conc.(ppb) in Raw Water**	**Atrazine Conc.(ppb) in Finished Water**
1	January	1/3/2000	3.1	2.7
	April	4/3/2000	1	0.6
	July	7/10/2000	3.3	3.9
	October	10/4/2000	3.6	3.2
2	February	2/7/2000	2.6	2.2
	May	5/1/2000	2.7	2.6
	August	8/14/2000	4.4	3.8
	November	11/13/2000	2.2	2.1
3	March	3/6/2000	0.9	0.7
	June	6/5/2000	3.6	7.2
	September	9/5/2000	3.4	2.8
	December	12/11/2000	2.7	2.4

Table 9 represents the data recorded for the three patterns in the year 2001.

Table 9:

For the Year 2001				
Pattern	**Month**	**Sample Date**	**Atrazine Conc.(ppb) in Raw Water**	**Atrazine Conc.(ppb) in Finished Water**
1	January	1/8/2001	2	1.8
	April	4/9/2001	0.26	0.23
	July	7/2/2001	0.59	0.40
	October	10/1/2001	0.55	0.40
2	February	2/5/2001	2.2	1.6
	May	5/7/2001	0.11	0.11
	August	8/6/2001	0.52	0.41
	November	11/12/2001	NA	NA
3	March	3/12/2001	0.36	0.31
	June	6/4/2001	0.25	0.13
	September	9/4/2001	0.51	0.50
	December	12/10/2001	NA	NA

Table 10 represents the data recorded for the three patterns in the year 2002.

Table 10:

For the Year 2002				
Pattern	**Month**	**Sample Date**	**Atrazine Conc.(ppb) in Raw Water**	**Atrazine Conc.(ppb) in Finished Water**
1	January	1/7/2002	0.20	0.08
	April	4/4/2002	1.53	0.72
	July	7/1/2002	2.58	2.82
	October	10/7/2002	**	1.10
2	February	2/4/2002	0.09	0.06
	May	5/13/2002	5.74	4.87
	August	8/5/2002	6.00	2.33
	November	11/4/2002	0.85	0.49
3	March	3/4/2002	0.07	0.11
	June	6/3/2002	4.90	4.73
	September	9/9/2002	2.91	1.70
	December	12/2/2002	0.20	0.03

Foot Notes: 1.nd - non-detect

2. ** - Sample bottles broken upon arrival at the lab

3. NA- Data Not Available

4. * - above detection limit

The concentrations of atrazine in the next set of tables (11, 12, and 13) are representative of the samples collected in the third or fourth week of every month for each study year for a period of three years.

Table 11 represents the data recorded for the next three patterns in the year 2000.

Table 11:

For the Year 2000				
Pattern	**Month**	**Sample Date**	**Atrazine Conc.(ppb) in Raw Water**	**Atrazine Conc.(ppb) in Finished Water**
1	January	1/24/2000	3.2	1.8
	April	4/17/2000	1.7	1.7
	July	7/17/2000	4.6	4.4
	October	10/16/2000	2.7	2
2	February	2/21/2000	1.7	1.6
	May	5/22/2000	3.2	3.2
	August	8/21/2000	4.4	3.6
	November	11/27/2000	2.8	2.1
3	March	3/20/2000	0.8	0.5
	June	6/19/2000	3.2	4.4
	September	9/18/2000	3	2.8
	December	12/11/2000	2.7	2.4

Table 12 represents the data recorded for the next three patterns in the year 2001.

Table 12:

For the Year 2001				
Pattern	**Month**	**Sample Date**	**Atrazine Conc.(ppb) in Raw Water**	**Atrazine Conc.(ppb) in Finished Water**
1	January	1/22/2001	2.4	2
	April	4/23/2001	0.47	0.41
	July	7/23/2001	0.27	0.17
	October	10/29/2001	0.42	0.23
2	February	2/26/2001	0.81	0.79
	May	5/21/2001	0.53	0.37
	August	8/20/2001	0.67	0.48
	November	11/26/2001		
3	March	3/26/2001	0.27	0.19
	June	6/25/2001	0.24	0.08
	September	9/17/2001	0.52	0.34
	December	12/10/2001		

Table 13 represents the data recorded for the next three patterns in the year 2002.

Table 13:

For the Year 2002				
Pattern	**Month**	**Sample Date**	**Atrazine Conc.(ppb) in Raw Water**	**Atrazine Conc.(ppb) in Finished Water**
1	January	1/22/2002	0.37	0.25
	April	4/15/2002	1.28	0.76
	July	7/22/2002	2.74	2.90
	October	10/21/2002	0.74	0.51
2	February	2/18/2002	0.07	0.04
	May	5/20/2002	5.57	3.45
	August	8/19/2002	2.77	2.59
	November	11/25/2002	0.16	0.11
3	March	3/18/2002	0.03	0.03
	June	6/17/2002	4.25	3.85
	September	9/23/2002	2.81	1.38
	December	12/16/2002	0.27	0.54

Foot Notes: 1.nd - non-detect

2. ** - Sample bottles broken upon arrival at the lab

3. NA- Data Not Available

4. * - above detection limit

The data obtained for the first three sampling patterns (Tables 8, 9,&10) from each of the three years 2000, 2001, and 2002 is analyzed for summary statistics in order to compare the individual mean for each of the patterns in a single year.

Summary Statistics for Table 8:

Pattern 1:

```
Atrazine.Con.ppb...in.Finished.Water
     Min:                              0.600000
 1st Qu.:                              2.175000
    Mean:                              2.600000
  Median:                              2.950000
 3rd Qu.:                              3.375000
     Max:                              3.900000
 Total N:                              4.000000
   NA's :                              0.000000
Std Dev.:                              1.421267
```

Pattern 2:

```
Atrazine.Con.ppb...in.Finished.Water
     Min:                             2.1000000
 1st Qu.:                             2.1750000
    Mean:                             2.6750000
  Median:                             2.4000000
 3rd Qu.:                             2.9000000
     Max:                             3.8000000
 Total N:                             4.0000000
   NA's :                             0.0000000
Std Dev.:                             0.7804913
```

Pattern 3:

```
Atrazine.Conc.ppb...in.Finished.Water
     Min:                              0.700000
 1st Qu.:                              1.975000
    Mean:                              3.275000
  Median:                              2.600000
 3rd Qu.:                              3.900000
     Max:                              7.200000
 Total N:                              4.000000
   NA's :                              0.000000
Std Dev.:                              2.770529
```

Summary Statistics for Table 9:

Pattern 1:

```
Atrazine.Conc.ppb...in.Finished.Water
     Min:                              0.2300000
 1st Qu.:                              0.3575000
    Mean:                              0.7075000
  Median:                              0.4000000
 3rd Qu.:                              0.7500000
     Max:                              1.8000000
 Total N:                              4.0000000
   NA's :                              0.0000000
Std Dev.:                              0.7327289
```

Pattern 2:

```
Atrazine.Conc.ppb...in.Finished.Water
     Min:                              0.1100000
 1st Qu.:                              0.2600000
    Mean:                              0.7066667
  Median:                              0.4100000
 3rd Qu.:                              1.0050000
     Max:                              1.6000000
 Total N:                              4.0000000
   NA's :                              1.0000000
Std Dev.:                              0.7880567
```

Pattern 3:

```
 Atrazine.Con.ppb...in.Finished.Water
     Min:                             0.1300000
 1st Qu.:                             0.2200000
    Mean:                             0.3133333
  Median:                             0.3100000
 3rd Qu.:                             0.4050000
     Max:                             0.5000000
 Total N:                             4.0000000
   NA's :                             1.0000000
Std Dev.:                             0.1850225
```

Summary Statistics for Table 10:

Pattern 1:

```
Atrazine.Con.ppb...in.Finished.Water
     Min:                              0.080000
 1st Qu.:                              0.560000
    Mean:                              1.180000
  Median:                              0.910000
 3rd Qu.:                              1.530000
     Max:                              2.820000
 Total N:                              4.000000
   NA's :                              0.000000
Std Dev.:                              1.171552
```

Pattern 2:

```
Atrazine.Conc.ppb...in.Finished.Water
     Min:                              0.060000
 1st Qu.:                              0.382500
    Mean:                              1.937500
  Median:                              1.410000
 3rd Qu.:                              2.965000
     Max:                              4.870000
 Total N:                              4.000000
   NA's :                              0.000000
Std Dev.:                              2.188902
```

Pattern 3:

```
Atrazine.Conc.ppb...in.Finished.Water
     Min:                              0.030000
 1st Qu.:                              0.090000
    Mean:                              1.642500
  Median:                              0.905000
 3rd Qu.:                              2.457500
     Max:                              4.730000
 Total N:                              4.000000
   NA's :                              0.000000
Std Dev.:                              2.197322
```

The data obtained for the next three sampling patterns (Tables 11, 12, &13) from each of the three years 2000, 2001, and 2002 is analyzed for summary statistics in order to compare the individual mean for each of the patterns in a single year. This is done by analyzing the samples collected in the third week of each month for each study period.

Summary Statistics for Table 11:

Pattern 1:

```
Atrazine.Conc.ppb...in.Finished.Water
     Min:                              1.70000
 1st Qu.:                              1.77500
    Mean:                              2.47500
  Median:                              1.90000
 3rd Qu.:                              2.60000
     Max:                              4.40000
 Total N:                              4.00000
   NA's :                              0.00000
Std Dev.:                              1.28938
```

Pattern 2:

```
Atrazine.Con.ppb...in.Finished.Water
     Min:                             1.6000000
 1st Qu.:                             1.9750000
    Mean:                             2.6250000
  Median:                             2.6500000
 3rd Qu.:                             3.3000000
     Max:                             3.6000000
 Total N:                             4.0000000
   NA's :                             0.0000000
Std Dev.:                             0.9322911
```

Pattern 3:

```
   Atrazine.Conc.ppb...in.Finished.Water
     Min:                               0.500000
 1st Qu.:                               1.925000
    Mean:                               2.525000
  Median:                               2.600000
 3rd Qu.:                               3.200000
     Max:                               4.400000
 Total N:                               4.000000
   NA's :                               0.000000
Std Dev.:                               1.602862
```

Summary Statistics for Table 12:

Pattern 1:

Atrazine.Conc.ppb...in.Finished.Water	
Min:	0.1700000
1st Qu.:	0.2150000
Mean:	0.7025000
Median:	0.3200000
3rd Qu.:	0.8075000
Max:	2.0000000
Total N:	4.0000000
NA's :	0.0000000
Std Dev.:	0.8709908

Pattern 2:

Atrazine.Conc.ppb...in.Finished.Water	
Min:	0.3700000
1st Qu.:	0.4250000
Mean:	0.5466667
Median:	0.4800000
3rd Qu.:	0.6350000
Max:	0.7900000
Total N:	4.0000000
NA's :	1.0000000
Std Dev.:	0.2177919

Pattern 3:

Atrazine.Conc.ppb.in.Finished.Water	
Min:	0.0800000
1st Qu.:	0.1350000
Mean:	0.2033333
Median:	0.1900000
3rd Qu.:	0.2650000
Max:	0.3400000
Total N:	4.0000000
NA's :	1.0000000
Std Dev.:	0.1305118

Summary Statistics for Table 13:

Pattern 1:

Atrazine.Conc.ppb...in.Finished.Water	
Min:	0.250000
1st Qu.:	0.445000
Mean:	1.105000
Median:	0.635000
3rd Qu.:	1.295000
Max:	2.900000
Total N:	4.000000
NA's :	0.000000
Std Dev.:	1.214647

Pattern 2:

Atrazine.Conc.ppb...in.Finished.Water	
Min:	0.040000
1st Qu.:	0.092500
Mean:	1.547500
Median:	1.350000
3rd Qu.:	2.805000
Max:	3.450000
Total N:	4.000000
NA's :	0.000000
Std Dev.:	1.736402

Pattern 3:

Atrazine.Conc.ppb...in.Finished.Water	
Min:	0.030000
1st Qu.:	0.412500
Mean:	1.450000
Median:	0.960000
3rd Qu.:	1.997500
Max:	3.850000
Total N:	4.000000
NA's :	0.000000
Std Dev.:	1.694048

Summary Statistics for the Year 2000(for Table 5):

Atrazine.Conc.ppb...in.Finished.Water	
Min:	0.500000
1st Qu.:	2.100000
Mean:	3.066667
Median:	2.700000
3rd Qu.:	3.750000
Max:	9.000000
Total N:	30.000000
NA's :	0.000000
Std Dev.:	1.844532

Summary Statistics for the Year 2001(for Table 6):

Atrazine.Conc.ppb...in.Finished.Water	
Min:	0.0400000
1st Qu.:	0.1900000
Mean:	0.5058621
Median:	0.3700000
3rd Qu.:	0.4800000
Max:	2.0000000
Total N:	32.0000000
NA's :	3.0000000
Std Dev.:	0.5468515

Summary Statistics for the Year 2002(for Table 7):

Atrazine.Conc.pb...in.Finished.Water	
Min:	0.030000
1st Qu.:	0.370000
Mean:	1.822308
Median:	1.100000
3rd Qu.:	2.900000
Max:	7.750000
Total N:	39.000000
NA's :	0.000000
Std Dev.:	1.832151

Summary Statistics for the first sample of each month in the Year 2000:

```
Min:  0.600000
 1st Qu.:  2.175000
    Mean:  2.850000
  Median:  2.650000
 3rd Qu.:  3.350000
     Max:  7.200000
 Total N: 12.000000
   NA's :  0.000000
Std Dev.:  1.705872
```

Summary Statistics for the first sample of each month in the Year 2001:

```
Min:  0.1100000
 1st Qu.:  0.2500000
    Mean:  0.5890000
  Median:  0.4000000
 3rd Qu.:  0.4775000
     Max:  1.8000000
 Total N: 12.0000000
   NA's :  2.0000000
Std Dev.:  0.6006376
```

Summary Statistics for the first sample of each month in the Year 2002:

```
Min:  0.030000
 1st Qu.:  0.102500
    Mean:  1.586667
  Median:  0.910000
 3rd Qu.:  2.452500
     Max:  4.870000
 Total N: 12.000000
   NA's :  0.000000
Std Dev.:  1.761778
```

Summary Statistics for the third sample of each month in the Year 2000:

```
Min:  0.500000
 1st Qu.:  1.775000
    Mean:  2.541667
  Median:  2.250000
 3rd Qu.:  3.300000
     Max:  4.400000
 Total N: 12.000000
   NA's :  0.000000
Std Dev.:  1.181262
```

Summary Statistics for the third sample of each month in the Year 2001:

```
Min:  0.0800000
 1st Qu.:  0.2000000
    Mean:  0.5060000
  Median:  0.3550000
 3rd Qu.:  0.4625000
     Max:  2.0000000
 Total N: 12.0000000
   NA's :  2.0000000
Std Dev.:  0.5616484
```

Summary Statistics for the third sample of each month in the Year 2002:

```
Min:  0.030000
 1st Qu.:  0.215000
    Mean:  1.367500
  Median:  0.650000
 3rd Qu.:  2.667500
     Max:  3.850000
 Total N: 12.000000
   NA's :  0.000000
Std Dev.:  1.430614
```

APPENDIX--C

STRATEGIC DIAGNOSTICS INC.

RaPID Assay Atrazine Test Kit

Intended Use

The RaPID Assay Atrazine Test Kit can be used as a quantitative, semi-quantitative or qualitative enzyme immunoassay (EIA) for the analysis of atrazine in water (groundwater, surface water, well water). For applications in other matrices please contact our Technical Service department. The RaPID Assay@ Atrazine Test Kit allows reliable and rapid screening for atrazine and related compounds, with quantitation, between 0.1 ppb and 5.0 ppb. The minimum detection level of the kit is 0.046 ppb (as atrazine.)

Test Principles

The Atrazine RaPID Assay@ kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of atrazine and related compounds. The sample to be tested is added, along with an enzyme conjugate, to a disposable test tube, followed by paramagnetic particles with antibodies specific to atrazine attached. Both the atrazine (which may be in the sample) and the enzyme labeled atrazine (the enzyme conjugate) compete for antibody binding sites on the magnetic particles. At the end of an incubation period, a magnetic field is applied to hold the paramagnetic particles (with atrazine and labeled atrazine analog bound to the antibodies on the particles, in proportion to their original concentration) in the tube and allow the unbound reagents to be decanted. After decanting, the particles are washed with Washing Solution.

The presence of atrazine is detected by adding the enzyme substrate (hydrogen peroxide) and the chromogen 3, 3', 5, 5' - tetramethylbenzidine). The enzyme labeled atrazine analog bound to the atrazine antibody catalyzes the conversion of the

substrate/chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of acid. Since the labeled atrazine (conjugate) was in competition with the unlabeled atrazine (sample) for the antibody sites, the color developed is inversely proportional to the concentration of atrazine in the sample.

NOTE: Color development is inversely proportional to the atrazine concentration.

Darker color = lower concentration
Lighter color = higher concentration

The determination of the atrazine level in an unknown sample is interpreted relative to the standard curve generated from kit standards after reading with a spectrophotometer.

Performance Characteristics

The Atrazine RaPID Assay will detect atrazine and related compounds to different degrees. Refer to the table below for data on several of these compounds. The Atrazine RaPID Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc.) positive results requiring some action should be confirmed by an alternative method.

The Atrazine RaPID Assay immunoassay test does not differentiate between atrazine and other related compounds. The table below shows compounds at the method detection limit (MDL) which is the lowest concentration of the compound that can be picked up in the assay. The limit of quantitation (LOQ) is an approximate concentration required to yield a positive result at the lowest standard, this is the lowest concentration of the compound that can be quantified in the assay. The IC50 is the concentration required to inhibit one half of the color produced by the negative control. It is also used to calculate cross-reactivity values to similar compounds.

Compound	MDL(ppb)	LOQ(ppb)	IC 50(ppb)
Atrazine	0.046	0.1	0.72
Propazine	0.033	0.1	0.74
Ametryn	0.053	0.05	0.39
Prometryn	0.054	0.09	0.64
Prometon	0.056	0.31	2.22
Desethyl Atrazine	0.062	0.45	3.21
Terbutryn	0.090	0.76	5.50
Terbutylazine	0.310	2.15	15.5
Simazine	0.340	0.68	4.90
Desisopropyl Atrazine	0.800	30.1	217
Cyanazine	1.0	> 10000	> 10000
6-Hydroxy Atrazine	1.1	20.6	148

The following compounds demonstrated no reactivity in the Atrazine RaPID Assay at concentrations up to 1000 ppb: aldicarb, aldicarb sulfoxide, aldicarb sulfone, alachlor, benomyl, butachlor, butylate, captan, carbaryl, carbendazim, carbofuran, 2,4-D. 1,3-dichloropropene, dinoseb, MCPA, metolachlor, metribuzin, pentachlorophenol, picloram, propachlor, terbufos, thiabendazole, and thiophanate-methyl. .

The presence of the following substances up to 250 ppm was found to have no significant effect on Atrazine Rapid Assay@ results: copper, nickel, sulfate, magnesium, calcium, nitrate, and thiosulfate. Humic acid, iron, sulfide, and sulfite were found to have no significant effect up to 100 ppm. In addition, sodium chloride concentrations up to 0.65 M showed no effect on results.

Precautions

- Training is strongly recommended prior to using the RaPID Assay test system. Contact Strategic Diagnostics for additional information.

- Treat atrazine, solutions that contain atrazine, and potentially contaminated samples as hazardous materials.

- Use gloves, proper protective clothing, and methods to contain and handle hazardous material where appropriate.

- Reagents must be added in a consistent manner to the entire rack. A consistent technique is the key to optimal performance. Be sure to treat each tube in an identical manner.

- Water samples should be at a neutral pH prior to analysis. Samples containing gross particulate should be filtered (e.g. 0.2 um AnotopTM 25 Plus, Whatman, Inc.) to remove particles.

- Store all test kit components at 2°C to 8°C C36°F to 46°F). Storage at ambient temperature 08°C to 27°C or 64°F to 81°F) on the day of use is acceptable. Test tubes require no special storage and may be stored separately to conserve refrigerator space.

- Allow all reagents to reach ambient temperature 08°C to 27°C or 64°F to 81°F) before beginning the test. This typically requires at least 1 hour to warm from recommended storage conditions.

- Do not freeze test kit components or expose them to temperatures above 100°F C39°C).

- Do not use test kit components after the expiration date.

- Do not use reagents or test tubes from one test kit with reagents or test tubes from a different test kit.

- Do not mix reagents from kits of different lot numbers.

- Use approved methodologies to confirm any positive results.

- Do not under any circumstances attempt to disassemble the base of the magnetic rack. Magnets will be violently attracted to each other. .

- Adequate sample number and distribution are the responsibility of the analyst.

- The photometer provided in the accessory kit requires electricity and comes with a 110V adapter. Adapters for 220V are available.

- Do not expose color solution to direct sunlight.

- Do not dilute or adulterate test reagents or use samples not called for in the test procedure; this may give inaccurate results.

- Tightly recap the standard vials when not in use to prevent evaporative loss.

Materials Provided

- .Antibody Coupled Paramagnetic Particles in buffered saline containing preservative and stabilizers.

  30 test kit: one 20 mL vial
  100 test kit: one 65 mL vial

- Enzyme Conjugate.

  30 test kit: one 10 mL vial

  100 test kit: one 35 mL vial

- Standards

  Three concentrations (0.1, 1.0, 5.0 ppb) of atrazine standards in buffered saline containing preservative and stabilizers are supplied. Each vial contains 4 mL.

- Control

  A concentration (approximately 3 ppb) of atrazine in buffered saline containing preservative and stabilizers. A 4 mL volume is supplied in one vial.

- Diluent/Zero Standard

  Buffered saline containing preservative and stabilizers without any detectable atrazine.

  30 test kit: one 10 mL vial

  100 test kit: one 35 mL vial

- Color Solution containing hydrogen peroxide and 3, 3', 5, 5' - tetramethylbenzidine in an organic base.

  30 test kit: one 20 mL vial

  100 test kit: one 65 mL vial

- Stop Solution containing a solution of 2 M sulfuric add.

  30 test kit: one 20 mL vial

  100 test kit: one 60 mL vial

- Washing Solution containing preserved deionized water.

  30 test kit: one 70 mL vial

  100 test kit: one 250 mL vial

- Polystyrene test tubes

30 test kit: one 36 tube box

100 test kit: three 36 tube boxes

- User's Guide

Rapid Assay Accessory Kit

Accessory equipment may be rented or pl1rchased from Strategic Diagnostics. See "Ordering Information" for the appropriate catalogue numbers.

The accessory kit contains the following items:

- Adjustable Volume Pipet
- Eppendorf TM Repeater Pipettor
- Electronic timer
- Portable balance capable of weighing 10 g (for soil samples)
- Vortex: mixer
- Magnetic separation rack
- RPA-I RaPID Analyzer (or equivalent spectrophotometer capable of reading 450 nm in a] mL sample size).

Other Items

- 12.5 mL Combitips for the Repeater pipettor -- for 0.25 mL to 1.25 mL dispensing volumes (5)
- Pipet tips for adjustable volume pipet (100-1000 uL)

NOTE: Order replacement Combitips and pipet tips separately.

Materials Required but Not Provided

- Protective clothing (e.g., latex: gloves)
- Absorbent paper for blotting test tubes
- Liquid and solid "Waste containers Marking pen
- Instructional video (optional)

Suggestions for Pipettor Use

- Practice using both pipettes (adjustable volume and Repeater pipettor) with water and extra tips before you analyze your samples. .

Use a new tip each time you use the Repeater pipettor to pipette a different reagent to avoid reagent cross-contamination. Tips can be rinsed thoroughly, dried completely and reused. By using the same tip to dispense the same reagent each time you can avoid cross contamination.

NOTE: Repeator tips should be changed periodically (after~ 10 uses) **since precision deteriorates with use.**

- Draw the desired reagent volume into the Repeater pipettor and dispense one portion of the reagent back into the container to properly engage the ratchet mechanism. If you do not do this, the first volume delivered may be inaccurate.
- To add reagents using the Repeater pipettor, pipette down the side of the test tube just below the rim.
- When adding samples and standard using the positive displacement pipettor, always pipette into the bottom of the tube without touching the sides or bottom of the tube.

- Use a new adjustable volume pipet tip each time you pipette a new unknown.

Assay Procedure

Prior to performing your first Rapid Assay@, please take time to read the package inserts in their entirety and review the videotape if available. On site training is strongly recommended for new users of this test system. please contact your account manager for further information.

Collect/ Store the Sample

The following steps explain how to properly collect and store your samples.

1. Samples should be collected in appropriately sized and labeled containers.

2. If testing soil samples, follow the SD I Sample Extraction Kit User's Guide or the appropriate tech bulletin, to properly collect and store your sample.

3. Water samples should be collected in glass VOC vials with minimal head space.

4. Samples should be tested as soon as possible after collection. If this is not possible, storage at *4°C* G9°F) is recommended to minimize evaporative losses. _

Set Up

1. Remove kits from refrigerator. All reagents must be allowed to come to room temperature prior to analysis. Remove reagents from packaging and place at room temperature at least 1 hour prior to testing.

2. Turn on the RPA-l or other spectrophotometer. The RPA-l should be warmed up for at least 30 minutes prior to the run.

3. Label five 12.5 mL Combitips "Conjugate", "Particles", "Wash", "Color" and "Stop". In addition, add the name of the compound you are testing for to each Combitip.

4. Remove nine clean blank test tubes for standards and control and one test tube for each sample (if testing in singlicate). Label the test tubes according to contents as follows.

Tube #	Contents
1	Negative control (replicate 1)
2	Negative control (replicate 2)
3	Standard 1 (replicate 1)
4	Standard 1 (replicate 2)
5	Standard 2 (replicate 1)
6	Standard 2 (replicate 2)
7	Standard 3 (replicate 1)
8	Standard 3 (replicate 2)
9	Control
10	Sample 1
11	Etc.

* Label at top of tubes to avoid interference with reading of tubes in photometer

Sample Extraction and Dilution

Water samples being tested at standard kit detection levels do not require extraction. Filtration may be necessary to remove gross particulate from the sample. If testing at levels higher than standard kit levels is desired, contact SDI for special instructions. Please follow the instructions from the SDI Sample Extraction Kit to prepare and dilute the soil extract prior to running the assay.

Perform the Test

1. Separate the upper rack from the magnetic base. Place labeled test tubes into the rack.

2. Add 200 uL of standards, control or samples to the appropriate tubes using the adjustable volume pipet with the dial set on 0200. The standards and control must be run with each batch of samples.

NOTE: Sample should be added to the bottom of the tube by inserting the pipet tip into the tube without touching the sides or the bottom of the tube. Take care not to contact sample with pipette tip once dispet15ed into bottom of the tube.

3. Using the Repeater Pipettor with the "Conjugate" tip attached and the dial set on "r', add 250 uL of Enzyme conjugate down the inside wall of each tube. (Aim the pipet tip 14" to *W'* below the tube rim or tube wall; deliver liquid gently to avoid splashback)

4. Thoroughly mix the magnetic particles by swirling (avoid vigorous shaking) and attach the" Particles" tip to the Repeater Pipettor. With the dial set on "'Z' add 500 uL of magnetic particles to each tube, aiming down the side of the tube as described above. Vortex, mixing each tube 1 to 2 seconds at low speed to minimize foaming. Pipetting of magnetic particles should be kept to 2 minutes or less.

5. Incubate 15 minutes at room temperature.

6. After the incubation, combine the upper rack with the magnetic base and press all tubes into the base; allow 2 minutes for the particles to separate.

7. With the upper rack and magnetic base combined, use a smooth motion to invert the combined rack assembly over a sink and pour out the tube contents.

NOTE: If the rack assembly inadvertently comes apart when lifting to pour out tube contents, recombine and wait an additional 2 minutes to allow particles to separate.

8. Keep the rack inverted and gently blot the test tube rims on several layers of paper towels. It is important to remove as much liquid as possible but do not bang the rack or you may dislodge the magnetic particles and affect the results.

9. Set the Repeater Pipettor dial to "4" and put on the tip labelled "Wash". Add 1 mL of Washing Solution down the inside wall. of each tube by using the technique described earlier. Wait 2 minutes and pour out the tube contents as described previously. Repeat this step one more time.

NOT E: T he number of washes and wash volume are important in ensuring accurate results.

10. Remove the upper rack (with its tubes) from the magnetic base. With the" Color" tip attached to the Repeater Pipet and the dial set to "'Z' add 500 uL of Color Reagent down the inside wall of each tube as described previously. Vortex 1 to 2 seconds (at low speed).

11. Incubate 20 minutes at room temperature. During this period, add approximately 1 mL of Washing solution to a clean tube for use as an instrument blank for "Results Interpretation".

12. After the incubation, position the Repeater pipettor at Setting" 2" and use the" Stop" tip to add 500 uL of Stop solution to all test tubes.

13. Proceed with results interpretation.

WARNING:

Stop solution contains 2M sulfuric acid Handle carefully.

Results Intetpretation

1. After addition of Stop Solution to the test tubes, results should be read within 15 minutes.

2. Wipe the outside of all antibody coated tubes prior to photometric analysis to remove fingerprints and smudges.

Photometric Interpretation U sing the RP A-I

1. The RP A - I photometer (provided in the Rapid Assay Accessory kit) can be used to calculate and store calibration curves. I t is preprogrammed with various RaPID Assay protocols. To obtain results from the Atrazine Rapid Assay@ on the RP A-I the following parameter settings are recommended:

Data Reduct: Lin. Regression
Xformation : Ln/LogitB
Read Mode : Absorbance
Wavelength : 450 nm

Units : PPB
Rgt Blk : 0

Calibrators:

# of Cals : 4

# of Reps : 2

Concentrations:

1: 0.00 ppb

#2:	0.10 ppb	
#3:	1.00 ppb	
#4:	5.00 ppb	
Range	:	0.05 - 5.00
Correlation	:	0.990
Rep. %CV	:	10%

NOTE: Prior to analysis the RPA-I User's Manual should be thoroughly reviewed for more detailed operation instructions.

2. Follow the instrument prompts to read the absorbance of all tubes:

Instrument Display	Operator Response
SELECT COMMAND	Press RUN
RUN PROTOCOL	Scroll using the YES( ) or NO( ) keys until the Desired protocol appears. Then press ENTER
SPL. REPLICATES (1-5)	Press 1 (for analysis of samples in singlicate.) Press ENTER
BLANK TUBE,	Insert blank tube
INSERT TUBE,	containing 1 ml wash solution
EVALUATING TUBE,	
REMOVE TUBE (Beep)	Remove tube
CAL # 1, REP. # 1,	Insert Tube # 1
INSERT TUBE,	
EVALUATING TUBE,	
REMOVE TUBE (Beep)	Remove tube

Follow prompts to read tubes.

NOTE: Tube order is important. The RPA-I expects to see the standards in ascending order, in duplicate, starting with the negative control.

Following evaluation of all standards, the instrument will display:

PRINTING DATA,	Data will print
PRINTING CURVE RPA1 User's Manual).	Curve will print only if programmed to print (See
CTRL # 1 REP # 1, INSERT TUBE, EVALUATING TUBE, REMOVE TUBE (Beep)	Insert Control Tube Remove Tube
EDIT CALIBRATORS YES/NO	Press NO (if editing is necessary press YE S and refer to the RPAl User's Manual).
SPL # 1 REP# 1 INSERT TUBE EVALUATING TUBE REMOVE TUBE (Beep)	Insert first sample tube Remove tube

Continue to follow prompts. After all samples have been read, press STOP.

Expected Results

- %CV (coefficient of variation) _ standard duplicates of 1QJ/o or less.

- Absorbance reading for the 0 ppb standard should be _ 0.8 and 2. 0 for all assays.
- Correlation (r) of 0.990 or greater for all assays.
- Kit control within range specified on vial.
- Absorbance of negative control and standards should be as follows:
- Negative Control> Std. 1> Std. 2> Std. 3.

3. Concentrations will be indicated for all samples on the RPA-I printout.

a) Samples with an "nd" and no concentration listed have an absorbance greater than the negative control; therefore, no concentration can be computed for these samples. Results must be reported as < 0.1 (Standard 1).

b) Samples with an "nd" next to a listed concentration have an estimated concentration below the minimum detection level of the test kit. Results must be reported as <0.1 (Standard 1).

NOTE: Any samples with Concentrations determined to be lower than Standard 1 (the limit of quantitation) must be reported as < 0.1 ppb.
Quantitation is not possible below this standard as this is outside the linear range of the assay.

c) Similarly, samples with a "hi" next to a listed concentration have an estimated concentration higher than Standard 3 and must be reported as > 5.0 ppb.

NOTE: In order to determine the concentration of
samples with Concentration greater than Standard 3, they must be subjected to repeat testing using a diluted sample. A ten- fold or greater dilution of the sample is recommended with an appropriate amount of atrazine diluent. T his additional dilution

must then be taken into account when calculating the concentration.

d) The concentration, as indicated on the printout, is multiplied by the appropriate dilution factor (if applicable) introduced in the procedure. For example, if the sample was diluted a total of 10x, the concentrations listed on the printout should be multiplied by 10 to determine the appropriate sample concentration.

Photometric Interpretation Using Other Photometers

Other photometers may also be used to interpret results obtained from the RP A-I photometer. It is important that the photometer be able to read absorbance at 450nm and that the instrument can read at a 1 mL fill volume.

Absorbances obtained from other spectrophotometers (reading at 450 nm) may be used to manually calculate sample concentrations as outlined below.

1. Calculate the mean absorbance for each of the three standards and the negative control.

2. Determine the standard deviation and %CV (coefficient of variation) of each standard and ensure %CV is less than 10% for each.

3. Calculate the %B/Bo for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the negative control and multiplying the results by 100.

4. Construct a standard curve by plotting the %B/Bo for each standard on the vertical logit (y) axis versus the corresponding analyte concentration on the horizontal logarithmic (x) axis on the graph paper provided in the test kit. Graph papers are specific for each method. Use only the graph paper supplied with each kit.

5. Draw the best straight line through all points. Using the %B/Bo of the sample, the concentration can be interpolated from the standard curve.

6. Multiply results by the appropriate dilution factor (if applicable) introduced in the procedure. For example, if the sample was diluted 10-fold to increase the detection levels of the kit then the results must be multiplied by 10. This dilution also changes the range of the assay (standards) by the same factor. So, if the 10 fold dilution were made, the range of the kit would now be 1.0 ppb to 50 ppb.

Limitations of the Procedure

The Rapid Assay Atrazine Test Kit is a screening test only. Sampling error may significantly affect testing reliability. Adequate sample number and distribution are the responsibility of the analyst.

Operation of the Repeater Pipet

To Set or Adjust Volume

To determine the pipetting volume, the dial setting (1-5) is multiplied by the minimum pipetting volume of the tip (indicated on the side of the Combitip, e.g. 1~100 uL.)

To Assemble Pipet Tip

Slide filling lever down until it stops. Then raise the locking clamp and insert the tip until it clicks into position. Be sure the tip plunger is fully inserted into the barrel before lowering the locking clamp to affix the tip in place.

To Fill Tip

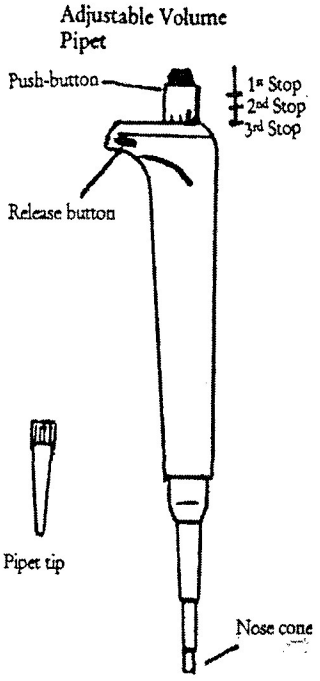
With tip mounted in position on pipet, immerse end of tip into solution. Slide filling lever upward slowly. Combitip will fill with liquid.

To Dispense Sample

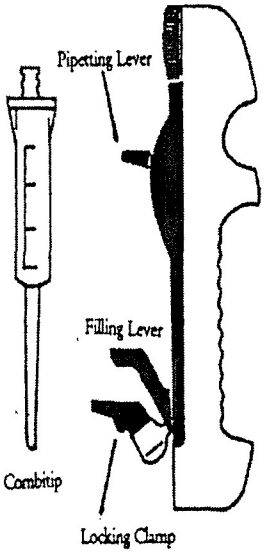
Check the volume selection dial to ensure pipetting volume. Place tip inside test tube so that tip touches the inner wall of tube. Completely depress the pipetting lever to deliver sample. NOTE: Dispense one portion of reagent back into the container to engage the ratchet mechanism and ensure accuracy.

To Eject Tip

Empty tip of any remaining solution into appropriate container by pushing filling lever down. Raise locking clamp upward, and remove the Combitip.



Repeater Pipet



Operation of the Adjustable Volume Pipet

To Set or Adjust Volume

Press release button on side of pipette and turn the push-button to adjust volume up or down. Volume setting is displayed on top of pipet. See kit instructions for appropriate setting. Pipet will accurately dispense volumes between 100 and 1000 uL.

To Assemble Pipet Tip

Gently push nose cone of pipet firmly into a pipet tip contained in the pipet tip rack.

To Withdraw Sample

Keep pipet almost vertical. With tip mounted in position on pipet, press push-button to 1st stop and hold it. Place tip at bottom of liquid sample and slowly release push-button to withdraw measured sample. Ensure that no air bubbles exist in the pipette tip. If bubbles exist, dispense sample and re-withdraw. Slide tip out along the inside of the vessel.

To Dispense Sample

Wipe any liquid from outside of tip taking care not to touch orifice. Place tip into tube, almost to the bottom, and slowly press push-button to 2nd stop. Hold push-button at 2nd stop when removing tip from tube.

To Eject Tip

Press push-button to 3rd stop. Tip is ejected.